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# CERTAIN EFFECTS UNDER IRRIGATION OF COPPER COMPOUNDS UPON CROPS*

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# Part I.—EXPERIMENTAL WORK

## INTRODUCTION

The region to which the studies described in this publication more particularly relate lies in southeastern Arizona in Greenlee and Graham counties and consists, first, of the Clifton-Morenci mining district and second, of the irrigated lands along the Gila River from twenty-five to sixty miles below. The Clifton-Morenci mining district is drained by Chase Creek into the San Francisco River, which in turn empties into the Gila. From the Gila, beginning at a point about twenty-five miles by channel below Clifton, irrigating waters are withdrawn for the use of the rich lands extending somewhat discontinuously from above San Jose to Fort Thomas, a distance of thirty miles. For about forty years, this up-stream mining district and the irrigated lands below have developed together from small beginnings into large industries.

Beginning with the initiation of smelting operations on the

San Francisco River in 1882, comparatively small amounts of mining detritus must have found their way into the irrigating water-supply. Following the discovery, in 1893, of immense deposits of low-grade sulphide ores in the district and the erection of concentrating plants to handle them, rapidly increasing quantities of fine slimes were discharged into the stream-flow, becoming noticeable in the irrigating waters of Graham County about the year 1900. Following the observation of their presence, various crop failures were attributed from time to time to the tailings, resulting finally in a request by the farmers of the district to the writer, for an examination of the facts relating to damage done by mining detritus to their irrigated crops.

### Solid Wastes

Following this request, the writer began a study of the problem in May, 1904, which resulted in the publication of Bulletin 53 of the Arizona Agricultural Experiment Station, September 20, 1906. This publication established the fact that irrigating sediments, in general, may be beneficial or harmful according to their composition and physical character and to the manner of their disposition in or upon the soil. If allowed to accumulate upon the surface of the soil in the form of more or less impervious silt-blankets, their influence, by limiting the supply of water and air to the soil, is notably harmful. In the case of the mining wastes from the Clifton-Morenci district, which are particularly plastic and "tight" in character, the damage done was found to be greater than that resulting from sediments arising from ordinary erosion. It was determined that the damage from these wastes, particularly to alfalfa and other crops which cannot receive constant and thorough cultivation, was of an increasingly serious character.

The farmers of Graham County, represented by one of their number, finally brought suit against the Arizona Copper Company, Limited, for discharging tailings into their irrigating water-supply. The case was decided in the District Court of Graham County in favor of the farmers, and an order was issued in November, 1907, effective May 1, 1908, restraining the mining companies from discharging "slimes, slickens or tailings" into

Chase Creek, the San Francisco River, or the Gila River. The case was appealed to the territorial Supreme Court where, however, the decision was confirmed in March, 1909. The case was again appealed by the Arizona Copper Company to the Supreme

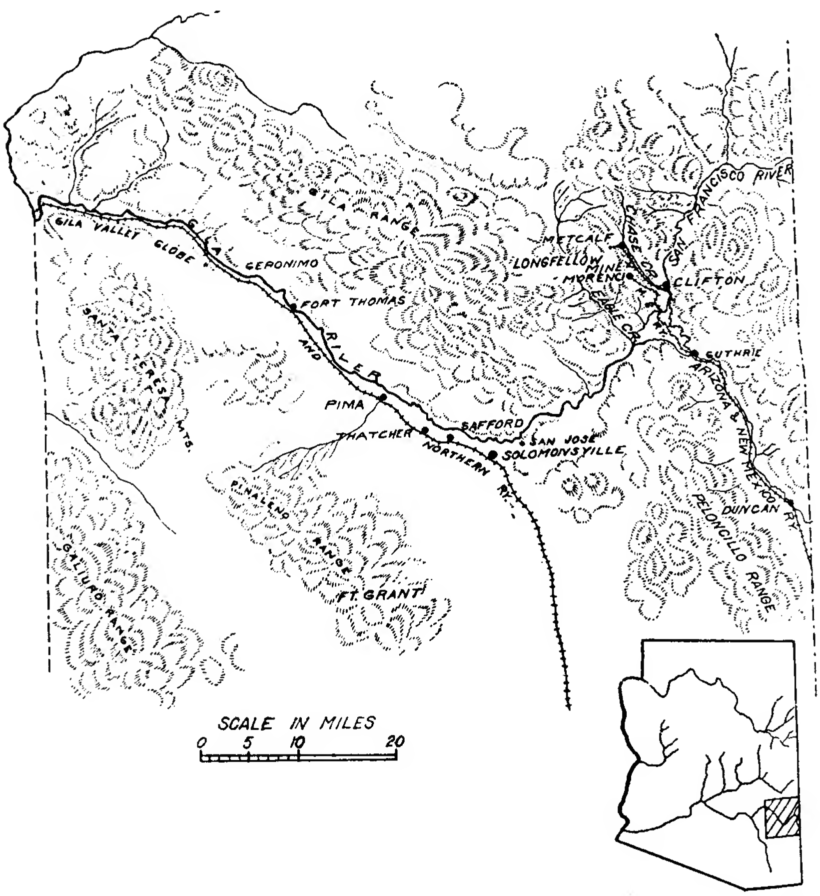


Fig. 1.—General map of the Clifton-Morenci and Gila River mining and irrigation district, Arizona

Court of the United States, where it was again and finally decided in favor of the farmers on June 16, 1913.

During and since the occurrences above mentioned, large quantities of solid wastes have been impounded by the copper companies in settling basins constructed for their storage in the

district. Recent investigations by the companies indicate a possibility that with copper at 15 cents a pound these stored tailings, which average about 0.85 per cent copper, may be profitably reworked.

In the long run, therefore, it may be found that an adjustment based upon a complete and impartial statement of facts relating to the tailings situation is beneficial both to the agricultural and to the mining interests concerned.

## Soluble Copper Compounds

Following the disposition of mining detritus, there remained the problem of soluble copper compounds which, in small but continuously appreciable quantities, find their way with waste waters into the stream-flow of the region. These compounds originate in the ores of the district and are, as in the case of the carbonates, directly soluble to a slight extent in drainage waters, especially in the presence of carbon dioxide. In other cases, the original ores are changed through the action of air into soluble substances which then escape downstream. Sulphide ores are thus oxidized in the presence of air into soluble copper sulphate. Inasmuch as it is well known that minute amounts of copper in solution are extremely toxic to plant roots directly exposed to them, some apprehension naturally existed as to the effects of these small amounts of copper salts escaping into the water-supply of an irrigated district.

In some respects, conditions were especially favorable here to the successful prosecution of a study of the foregoing question. The irrigated lands are at a distance of twenty miles or more from the smelters, so that injurious gases could not complicate effects upon irrigated crops. There are, also, only traces of other toxic metals to be found within the district—more particularly, arsenic, antimony, and zinc. Injurious effects due to the possible toxic action of compounds originating in the mines are therefore limited to copper.

Scientific study relating to toxic effects of copper upon plants under varying conditions has thoroughly established not only the fact that copper compounds are extremely toxic to plants when they obtain entry to their tissues, but also that various

agencies standing between these poisonous salts and the living plant tend to prevent injury.[1] Soluble copper compounds, for instance, react with carbonate of lime, commonly abundant in soils of the arid region, to form the solid carbonates of copper. The partly decomposed silicates of these soils also precipitate soluble compounds of copper and mask their toxic character. Organic matter in the soil likewise holds large quantities of copper in comparatively harmless combinations. Through physical attraction or *adsorption*, soluble copper compounds enter into weak combination with fine soil particles and toxic effects are thereby greatly lessened. In the presence, also, of other soluble salts, such as the various forms of "alkali" commonly found in the soils of the region, the toxicity of copper compounds is enormously lessened.

The investigations recorded in this publication include: (1) Observations upon the distribution of copper in mining wastes, in irrigating waters, in soils and soil waters, in the plants, and in the animal life of the region. (2) The development of accurate methods for the determination of minute amounts of copper in all situations where they may occur. (3) Plant cultural work with waters and in soils in the presence of varying proportions of copper and under varying conditions. (4) A careful analytical study of the results of such cultures in order to determine the symptoms of poisoning and the distribution of copper throughout poisoned plants; and to identify, if possible, the particular parts of plants and tissues injured by copper. (5) A physiological study of plant reactions with copper. (6) Field studies for the purpose of relating the results of laboratory investigations to the question of economic injury done by copper salts to irrigated crops.

By reason of interruptions due to other duties, it has required a long time to mature this investigation to the point where it seems sufficiently complete for publication. This delay, however, has given perspective to the work and, especially, opportunity to verify earlier conclusions as applied to field conditions.

The writer is indebted for painstaking analytical work to Messrs. R. G. Mead, Edward E. Free, Dr. W. H. Ross and

[1] See Bibliography, pp. 487–488, references 1, 8, 14, 15, 16, 19, 34, 51.

C. N. Catlin, associated with the Arizona Agricultural Experiment Station from time to time; and to the helpful advice of Dr. Howard S. Reed, of the University of California Graduate School of Tropical Agriculture, in connection with the physiological part of the work herein described. The publication, also, has been criticized to its advantage by Dr. C. B. Lipman of the University of California.

## DISTRIBUTION OF COPPER COMPOUNDS THROUGHOUT THE CLIFTON-MORENCI AND GILA RIVER MINING AND IRRIGATION DISTRICTS

### Sources of Copper

The original source of the copper found in this district, according to Lindgren,[2] is a Cretaceous or early Tertiary intrusion of acidic porphyries to which, in the Clifton-Morenci district, all ore deposits may be finally referred. The original porphyries contain as little as 0.02 per cent of copper ore in the form of chalcopyrite. Under the influence of superheated waters emanating from the porphyry, this chalcopyrite, together with other metallic compounds, was carried out from the molten intrusive mass into adjoining strata and there deposited, especially along fissures, in the form of concentrated masses or veins of chalcopyrite and other minerals. Through erosion these deposits were afterward subjected to atmospheric oxidation, followed by downward percolation and a period of secondary enrichment due to numerous reactions mainly between the oxidized compounds of copper and other minerals present.

In limestones and shales, these processes resulted in the formation of oxidized ores containing azurite, malachite, chrysocolla, and cuprite. In porphyry, the main final result was chalcocite or copperglance, the principal constituent of the sulphide ores of the Clifton-Morenci district.

In general, therefore, the metasomatic changes associated, first, with superheated waters arising from the original intrusion of molten porphyry and, second, with meteoric waters percolating

[2] U. S. Geological Survey, Professional Paper No. 43, 1905.

downward with oxidizing effects through copper-bearing rocks, have brought copper from a concentration of possibly less than 0.02 per cent in the original porphyry through every degree of richness to the condition in some cases of pure copper.

## Processes by which Copper is Added to the Water-Supply

To a slight extent, drainage waters from the ore deposits and from the mines, containing considerable amounts of copper in solution, find their way downstream. But by far the larger part of the copper which gets into the irrigating supply is derived from the ores and tailings which, in the concentrators, on the dumps, and finally in the river itself, are subjected to the action of atmospheric oxygen, and water containing carbon dioxide and various salts in solution. The residual chalcocite in tailings from sulphide ores thus reacts with oxygen from the air and yields copper sulphate in solution. This, in turn, reacts with the excess of bicarbonate of lime ordinarily contained in the waters of the San Francisco and Gila rivers. The resulting basic carbonate of copper is notably soluble in water containing carbon dioxide and certain of the various salts commonly found in river waters. The residues of carbonates of copper in oxidized ores are directly dissolved in waters containing carbon dioxide and certain soluble salts.

Along with minute quantities of copper thus dissolved and carried forward, pass the solid residues discharged from the concentrators—solid wastes which find their way, unchanged, downstream and finally upon the soils of irrigated fields. At this point begins another and very important series of reactions between dissolved copper compounds and the soil, tending in general to withdraw copper from its solutions and precipitate it in the form of less harmful solid compounds. These are briefly referred to above and will be discussed more in detail further on in this paper. Opposing these precipitations of copper are those solvents which tend to maintain this metal in soluble form in small quantities in the soil. Chief of these is carbon dioxide, which is always present in agricultural soils in significant quantities. Of interest in this connection is the fol-

### TABLE I

SOLUBILITIES OF COPPER COMPOUNDS

| Compound | Solvent | Cu dissolved, parts per million | Reference |
|---|---|---|---|
| Malachite $CuCO_3.Cu(OH)_2$ | Water containing 0.12% carbon dioxide | 29.0–31.0 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1367 |
| Precipitated basic copper carbonate | Pure water | 1.5 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1370 |
| Precipitated basic copper carbonate | Water containing 0.12% carbon dioxide | 34.8 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1370 |
| Precipitated basic copper carbonate | Water containing 0.13% carbon dioxide and 0.01% sodium chloride | 36.0 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1371 |
| Precipitated basic copper carbonate | Water containing 0.13% carbon dioxide and 1.0% sodium chloride | 58.0 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1371 |
| Precipitated basic copper carbonate | Water containing 0.12% carbon dioxide and 0.01% sodium sulphate | 37.0 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1372 |
| Precipitated basic copper carbonate | Water containing 0.12% carbon dioxide and 1.0% sodium sulphate | 58.0 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1372 |
| Precipitated basic copper carbonate | Water containing 0.12% carbon dioxide and 0.01% sod. carbonate | 10.0 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1372 |
| Precipitated basic copper carbonate | Water containing 0.12% carbon dioxide and 1.0% sod. carbonate | 0.7 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1372 |
| Precipitated basic copper carbonate | Water containing 0.12% carbon dioxide and 0.2% calcium sulphate | 36.0 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1372 |
| Precipitated basic copper carbonate | Water containing 0.12% carbon dioxide and 0.11% calc. carbonate | 1.4 | E. E. Free, Journ. Am. Chem. Soc., XXX, 9, p. 1372 |
| Copper sulphide; $CuS$ | Oxygen-free water | 0.09 | W. H. Ross, MSS |
| Chalcopyrite $CuFeS_2$ | Pure water | measurable amounts | U. S. Geol. Survey Monograph XLVII, p. 1107 |
| Chalcopyrite $CuFeS_2$ | Sodic sulphide | Amt. not stated | U. S. Geol. Survey Monograph XLVII, p. 1106 |
| Malachite | "Insoluble in water, slightly soluble in water charged with carbon dioxide." | | Moissan 5, p. 167 |
| Chrysocolla $Cu\,Si\,O_3.n\,H_2O$ | "Somewhat soluble in water with carbon dioxide" | | Lindgren, U. S. G. S. Prof. paper 43, p. 188 |
| Cupric sulphide $CuS$ | Water | 1 to 950,000 | Comey, Dict. Solubilities, p. 139 |
| Cuprite $Cu_2O$ | "Insoluble in water" | | Comey, Dict. Solubilities, p. 137 |
| Cupric oxide $CuO$ | "Insoluble in water" | | Comey, Dict. Solubilities, p. 137 |

lowing table of solubilities of various compounds of copper in different solvents, made up from different sources of information. The exact determinations of solubility by E. E. Free and W. H. Ross were made to obtain data needed in this investigation.

This table indicates that the carbonates and the silicate (chrysocolla) of copper, which are the compounds in which the metal must largely occur in the soil, are notably soluble in aqueous solutions of carbon dioxide.[3] Large amounts of sodium chloride and sodium sulphate increase the solubility of precipitated basic copper carbonate. In pure water, copper compounds, so far as observed, are but slightly soluble. Fluctuations in the content of carbon dioxide and of soluble salts in soil waters, and variations in the character of the soluble salts, are shown to affect the copper content of such waters.

In brief, the final effect upon plant roots of copper in the soil is the complex resultant of many opposing influences tending, on the one hand, to remove copper from solution, and, on the other, to maintain it in toxic soluble form. *Observations on the soil* usually fail to give satisfactory evidence as to the toxic or non-toxic effects to be expected from small percentages of copper that may be present. *Direct chemical and physiological studies of plants* afford much more satisfactory information. This mode of attack has been employed considerably in this investigation.

In view of the general tendency in nature to hinder the movements of copper in soils and to convert it into its insoluble forms, and independently of any tendency of the plant itself to assimilate or to reject copper, we should expect to find relatively small amounts of this element in plant tissues.

The following analytical determinations of copper in ores and tailings were made in samples carefully collected by the writer throughout the district studied. In all cases, the copper was determined electrolytically, manipulations of great delicacy having been developed for the determination of the minute amounts of copper often encountered. A full statement of the methods of preparing samples for analysis, and of determining

[3] Sullivan has shown that powdered silicates react with copper sulphate to withdraw copper from solution; and that this copper will then be redissolved by a solution of potassium sulphate. U. S. Geol. Survey, Bull. 312, 1907.

copper in ores, tailings, waters, soils, and organic materials, is to be found under "Methods of Analysis" in the appendix to this paper. For convenience in comparing widely variable amounts in the samples examined, the copper content is given in parts per million of substance. Parts per million may be reduced to percentages by moving the decimal point four places to the left. For instance, 11,600 parts per million is equal to 1.16 per cent.

TABLE II

COPPER IN ORES AND TAILINGS FROM THE CLIFTON-MORENCI MINING DISTRICT

| Sample No. and date | Description of sample | Condition and weight taken, grams | Cu found, grams | Cu parts per million |
|---|---|---|---|---|
| 3491 May 23, '04 | One day's run of sulphide ore from from A. C. Co.'s mill in Clifton | 1 air-dry | .03195 | 31,950 |
| 3303 May 23, '04 | Sulphide tailings, point of discharge from A. C. Co.'s mill, Clifton | 1 water-free | .00935 | 9,350 |
| 3438 June 28, '05 | Sulphide tailings, point of discharge from A. C. Co.'s mill, Clifton | 1 water-free | .0116 | 11,600 |
| 3499 June 28, '05 | Sulphide tailings at Clifton coming from Longfellow mill | 1 water-free | .00725 | 7,250 |
| D. C. Co.'s Records May 20, '04 | Fine sulphide tailings at Morenci | | | 10,000 |
| 3492 May 23, '04 | One day's run of oxidized ore from A. C. Co.'s mill at Clifton | 1 air-dry | .0553 | 55,300 |
| 3304 May 23, '04 | Oxidized tailings, point of discharge from A. C. Co.'s mill, Clifton | 1 water-free | .0225 | 25,500 |
| 3439 June 28, '05 | Oxidized tailings, point of discharge from A. C. Co.'s mill, Clifton | 1 water-free | .0268 | 26,800 |
| 3500 June 27, '05 | Oxidized tailings, point of discharge from Shannon C. Co.'s mill, Clifton | 1 water-free | .0114 | 11,400 |
| 3309 May 26, '04 | Milky sediments, pure tailings from Montezuma Canal, Solomonville | 2 water-free | .01725 | 8,625 |
| 3486 June 11, '05 | River sediments with tailings from Montezuma Canal, Solomonville | 2 water-free | .0067 | 3,350 |
| 3737 Feb. 22, '07 | High river sediments with muddy tailings from Montezuma Canal, Solomonville | 15.8 water-free | trace | trace |
| 6342 Mar. 4, '16 | Floodwater sediments from Montezuma Canal, Solomonville; tailings from Mogollon | .5899 | .000085 | 53 |

A point of interest to both mine owners and farmers in table II is the large proportion of copper that was discarded with tailings at the time the samples were taken. This loss, so far as these figures show, may amount to almost one-third of the copper

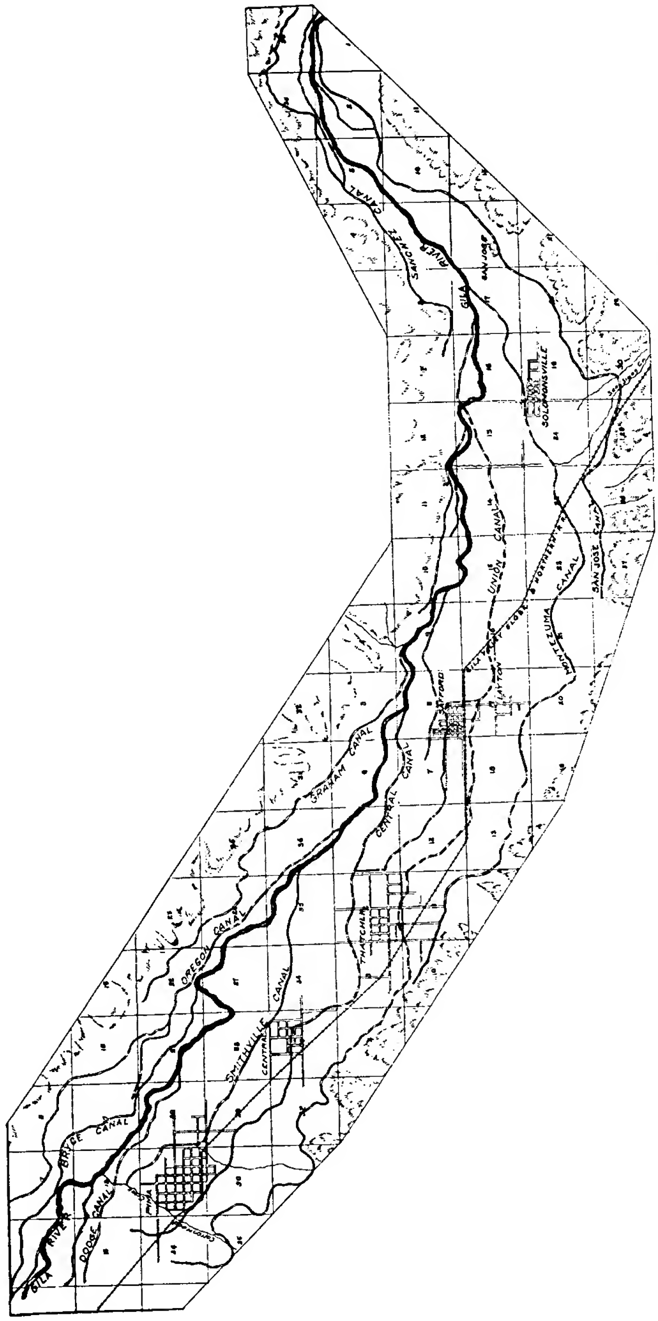


Fig. 2.—Detail map of the Gila River irrigation district, in Graham County, Arizona.

in low-grade sulphide ores (No. 3491, No. 3303), and to nearly one-half in the richer oxidized ores (No. 3492, No. 3304, No. 3439). By far the larger portion of tailings produced, however, are from low-grade sulphide ores, the wastes from which therefore predominate, formerly imparting to river waters the whitish

TABLE III

DISSOLVED COPPER IN RIVER, IRRIGATING, AND GROUND WATERS BELOW THE CLIFTON-MORENCI DISTRICT

| Sample No. and date | Description of sample | Condition and amount taken in cc. | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 3438 June 28, '05 | Water mixed with sulphide tailings from A. C. Co.'s mill, Clifton | 500 | .0009 | 1.80 |
| 3439 June 28, '05 | Water mixed with oxidized tailings from A. C. Co.'s mill, Clifton | 500 | .0018 | 3.60 |
| 3309 May 26, '04 | Montezuma Canal water at Solomonville; slight rise in river | 2000 | .0016 | .80 |
| 3486 June 11, '05 | Montezuma Canal at Solomonville, small flood | 6000 | .0015 | .25 |
| 3622 June 25, '06 | Montezuma Canal at Solomonville, head waters clear | 9000 | .00095 | .11 |
| 3737 Feb. 22, '07 | Montezuma Canal at Solomonville, medium flood | 14000 | .0403 | 2.88 |
| Tailings shut out of water supply May 1, 1908. | | | | |
| 4011 Jan. 3, '09 | Water from Montezuma Canal at Solomonville | 4000 | .00031 | .08 |
| 4029 Apr. 12, '09 | Montezuma Canal at Solomonville | 3700 | .0003 | .08 |
| 6342 Mar. 4, '16 | Montezuma Canal at Solomonville, high water | 1000 | .00003 | .03 |
| 3986 Jan. 2, '09 | Water from C. & A. Ditch, Bisbee mine waters | 3500 | .00039 | .11 |
| Records of Cananea C. C. Co. Jan. 4, '14 | Water from creek below concentrator | | | 2.1 |
| 3504 Aug. 19, '05 | Water from Geo. Olney's well, 30 ft. deep, east of Safford, under Montezuma Canal | 7000 | .0037 | .53 |
| 4012 Jan. 3, '09 | Water from Wilson's well, one-half mile west of Solomonville under San Jose Canal | 3500 | less than .00001 | less than .003 |
| 3526 | Water from University well, Tucson, 95 ft. deep, tapping Rillito underflow | 7000 | none | none |

appearance characteristic of this material. It is of interest to note in this connection that in one instance observed the tailings almost completely maintained their richness in copper between

Clifton and Solomonville. At Clifton, May 23, 1904, the principal discharge of sulphide tailings (3303) was observed carrying 0.93 per cent of copper. At Solomonville, three days later, the Montezuma ditch-water sediments (No. 3309), mostly of this same material, carried 0.86 per cent of copper, indicating the persistence with which the copper accompanies the wastes, with which it is associated, downstream and upon underlying irrigated lands.

TABLE IV

COPPER IN SOILS IRRIGATED WITH TAILINGS WATERS

| Sample No. and date | Description of sample | Condition and weight taken, grams | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 3435 May 25, '04 | Top 5 in. sedimentary soil (Fred Thorstison), upper end alfalfa field west of Safford, under Montezuma Canal | 96.7 water-free | .020 | 207 |
| 3434 June 10, '05 | Top 5 in. sedimentary soil (Geo. Olney), upper end alfalfa field east of Safford, under Montezuma Canal | 96.8 water-free | .0199 | 205 |
| 3501 Aug. 19, '05 | Soil in place at 4 ft. depth beneath No. 3434 | 96.2 water-free | .0021 | 22 |
| 3436 June 25, '05 | Top sedimentary soil (Wm. Gillespie), upper end of test alfalfa field west of Solomonville, under Montezuma Canal | 96.7 water-free | .0192 | 199 |
| 3437 June 25, '05 | Soil in place, no sediments at surface of lower end of field near No. 3436 | 94 water-free | .0028 | 30 |
| 3502 Aug. 19, '05 | Soil in place at 4 ft. depth beneath No. 3437 | 96.1 water-free | .001 | 10 |
| 2381 June 5, '00 | Surface 12 in. from garden near Pima, Ariz., beyond tailings deposits | 100 air-dry | faint trace | trace |
| 3522 Oct. 25, '05 | Top 4 in. sedimentary soil upper end of alfalfa field, Station farm near Phoenix, under Grand and Maricopa canals | 95 water-free | .0003 | 3 |
| 3521 Oct. 25, '05 | Deep soil, no sediments, Station farm near No. 3522 | 95 water-free | .0003 | 3 |
| 2763 Nov. 11, '01 | Surface 12 in. from orange orchard north of Phoenix, under Arizona Canal | 100 air-dry | faint trace | trace |
| 1890 Apr. 20, '01 | Surface 15 in. from cultivated field west of Tempe, under Tempe Canal | 100 air-dry | faint trace | trace |
| 2830 Jan. 19, '00 | Surface 12 in. from orange orchard northeast of Phoenix, under Arizona Canal | 100 air-dry | none | none |

Table III is of interest because it reveals quantities of dissolved copper in irrigating and in ground waters sufficient, under proper conditions, in water cultures, to produce toxic effects upon plants.[4] It is noteworthy, however, that, following the order of the court, effective May 1, 1908, prohibiting the introduction of tailings into the water-supply, the amount of dissolved copper in Montezuma canal waters greatly decreased, due to the decrease in quantity of sulphides whose oxidation affords the supply of dissolved copper. Other water-supplies also are found to contain similar amounts of copper, as the Calumet and Arizona mine waters, used for irrigation below Bisbee. As stated above, however, in the soil itself the toxic action of such copper solutions is enormously decreased. Naturally, the question arises as to the possibility of toxic effects in using such waters upon cultivated soils. This is discussed on subsequent pages. The proportions of copper (0.003 to 0.53 parts in 1,000,000 of water) found in the drainage beneath this irrigated district indicate that not all of the copper applied in irrigation remains in the soil. University well water at Tucson was observed to be free from this element.

Soils Nos. 3435, 3434, and 3436 show maximum amounts of copper, inasmuch as they are composed to a considerable extent of tailings. The soils in place beneath these sediments, Nos. 3501 and 3502, contain much less, yet noticeable amounts of copper, most of which is retained where it first comes in contact with the top soil. It is of interest to note that the surface sediments and the deep soils of the Experiment Station farm near Phoenix, Arizona, irrigated from an entirely different watershed, also contain small but weighable amounts of copper. This was probably derived from mines at Globe and Jerome, Arizona, whose wastes have found their way into the drainage which supplies irrigation for Salt River Valley. The quantities observed, however, three parts copper per million of soil, are negligible. Other soils from Salt River Valley also show traces of copper.

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[4] See Bibliography, p. 487, references 5, 18.

### TABLE V

MISCELLANEOUS SOILS UNAFFECTED BY MINING DETRITUS

| Sample No. and date | Description of sample | Condition and weight taken, grams | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 2375 June 5, '00 | Surface 12 in. from new ground near Safford, recently placed under Montezuma Ditch | 100 air-dry | none | none |
| 2253 Jan. 3, '00 | Surface 12 in. university ground, Tucson | 100 air-dry | none | none |
| 3503 May 9, '05 | Surface 12 in. virgin unirrigated soil, Colorado Valley bottom near Yuma | 100 air-dry | none | none |

These determinations, made in widely separated localities, indicate the absence of copper in soils which are not immediately under the influence of mining detritus.

### TABLE VI

COPPER IN VEGETATION FROM UPPER GILA VALLEY FARMS

| Sample No. and date | Description of sample | Condition and weight taken, grams | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 3505 Aug. 19, '05 | Alfalfa, before blooming, from upper end of Geo. Olney's field east of Safford, under Montezuma Ditch | 1206 air-dry | .0062 | 5.10 |
| 3512 Aug. 19, '05 | Alfalfa from bale grown in Layton (M. B. Steele) under Montezuma ditch | 1359 air-dry | .0077 | 5.70 |
| 3507 Aug. 20, '05 | Corn in bloom, leaves only, grown in Layton (Jas. Welker), under Montezuma Ditch | 545 air-dry | .0033 | 6.10 |
| 3509 Aug. 19, '05 | Wheat from stack, stalk and grain, grown in Layton (M. B. Steele), under Montezuma Ditch | 1125 air-dry | .0027 | 2.40 |
| 3513 Sept. 19, '05 | Mistletoe, growing on willow 25 ft. above ground, one mile east of Safford, under Montezuma Ditch | 1245 air-dry | .0094 | 7.60 |
| 3739 | Alfalfa seed, crop of 1906, grown near Pima under Smithville Ditch | 782 water-free | .0026 | 3.33 |
| 3741 | Alfalfa seed (Wm. Gillespie), crop of 1906, grown near Solomonville, under Montezuma Ditch | 843 water-free | .0023 | 2.72 |
| 3780 | Shelled corn, crop of 1906, grown at Solomonville, under Montezuma Ditch | 932 water-free | .0004 | .43 |
| 3740 | Shelled corn, crop of 1906, grown at Solomonville, under Montezuma Ditch | 874 water-free | .0008 | .73 |
| 3738 | Shelled corn, crop of 1906, grown near Pima, under Smithville Ditch | 1092 water-free | trace | trace |

The prevalence of small amounts of copper in vegetation throughout this locality is shown by the figures in table VI. Samples of corn and alfalfa contained comparable quantities of copper, which, however, were exceeded by the amount found in a sample of mistletoe growing on a willow fully twenty-five feet above the ground. This is due chiefly to the perennial character of mistletoe which, therefore, has more time to accumulate copper. It is interesting to note also that seeds of alfalfa and corn contain less copper than corresponding foliage. Corn leaves were observed to contain 6.1 parts of copper per million parts of air-dry substance, while grain from the same locality contained from 0.73 to 0.43 parts. Alfalfa seed contained about one-half as much copper as the stalks and leaves, while wheat hay carrying a large proportion of grain showed a low proportion of copper. These facts are probably connected with transpiration.

TABLE VII

COPPER IN VEGETATION FROM OTHER LOCALITIES

| Sample No. and date | Description of sample | Condition and weight taken, grams | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 3508 Aug. 25, '05 | Alfalfa hay, station farm near Phoenix (two samples) | 2109 air-dry | .0021 | 1.00 |
| | | 1408 air-dry | .0031 | 2.20 |
| 3516 Oct. 25, '05 | Alfalfa, before blooming, station farm near Phoenix | 1106 air-dry | .0011 | 1.00 |
| 3515 Oct. 4, '05 | Alfalfa hay, Colorado bottom, Yuma date orchard | 1262 air-dry | none | none |
| 3517 May, 1905 | Barley hay, station farm near Phoenix | 1304 air-dry | .0002 | .15 |
| 3518 Oct. 25, '05 | Corn, leaves only, station farm near Phoenix | 595 air-dry | .0005 | .84 |
| 3519 Oct. 14, '05 | Corn, leaves only, grown on Rillito near old Fort Lowell | 284 air-dry | .0018 | 6.30 |
| 3529 Dec. 27, '05 | Corn, leaves and bloom, same field as No. 3519 | 1132 air-dry | .0015 | 1.32 |
| 3520 Oct. 14, '05 | Mistletoe from cottonwood 30 ft. above ground, old Fort Lowell, near Tucson | 1160 air-dry | .001 | .85 |
| 3989 Dec. 31, '08 | Young (5 mos. old) alfalfa roots from C. & A. ranch irrigated with mine waters containing copper, from Bisbee | 2.12 air-dry | .0001 | 47.00 |
| 3990 | Corn roots from C. & A. ranch irrigated with mine waters containing copper, from Bisbee | 16.7 air-dry | .00025 | 15.00 |

which is maximum in leaves and quantitatively small in the fruiting parts of a plant. Additional evidence of this fact is shown in poisoned corn plants, which are discussed on a subsequent page.

Comparing the data of table VII with those of table VI, it is evident that, excluding corn and alfalfa irrigated with C. & A. mine waters, in every case except that of one sample of corn from old Fort Lowell (No. 3519) the copper in crops grown on Gila Valley farms is much in excess of that in plants coming from elsewhere for the same classes of material. The presence of appreciable amounts of copper in samples of alfalfa, corn, barley, and mistletoe also accords with the fact that the soils in which they were grown receive the drainage from copper-bearing watersheds. The one exception, at Yuma (No. 3515) where no trace of copper could be found either in alfalfa or in soil (No. 3503), indicates that these alluvial river deposits, which have been subjected annually to the leaching action of enormous quantities of flood waters, have been prevented from accumulating appreciable quantities of copper.

## Copper in the Flesh and Bones of a Pig

In order to follow the copper as far as possible in its transmigrations, a five-months-old pig that had been born and brought up in an alfalfa pasture near Solomonville under the Montezuma Ditch, was killed and portions of the flesh and bones were taken for examination, with the following results:

| Sample No. and date | Description of sample | Condition and weight taken, grams | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 3779 May 7,'07 | Liver, heart, and rib meat | 917 fresh | .0053 | 5.78 |
| 3778 May 7,'07 | Ribs and rib meat | 998 fresh | .00006 | .06 |

The largest amount of copper was found in portions of liver, heart and rib meat, only minute amounts being present in the bony material. In this connection, it is stated that about two parts of copper have been observed in one million parts of human liver; ten parts in human kidneys, and as much as fifty parts

in sheep's liver.[5] Human food, however, is commonly contaminated with copper compounds, which account for its presence in the human body.

*In brief,* the observations detailed above have shown the successive positions of copper in the original ores of the Clifton-Morenci district; in the tailings wastes from these ores, in suspension and in solution in river waters exposed to milling operations; in soils irrigated with these waters; in the ground waters beneath these soils; in vegetation growing upon them; and even in the animal life of the region. It is of interest to observe, first, the concentration through natural processes of small amounts of copper in the original rocks into the form of rich ores; and, second, the reversal, through human agencies, of this process, and the dilution of copper values till, in vegetation and in animal life, but traces of the metal can be detected.

## DISTRIBUTION OF COPPER IN PLANTS WITH ROOT SYSTEMS EXPOSED TO COPPER COMPOUNDS

### Corn Plants Grown in Soils Containing Copper

In order to determine accurately the distribution of copper throughout a typical crop plant, thereby locating if possible the points at which injury may occur from copper compounds in the soil, three lots of corn plants were examined in detail. Two of these were grown (August 3 to November 13, 1907) in pots containing thirty-eight pounds of sandy loam soil very thoroughly mixed with 0.01 and 0.025 per cent of copper in the form of freshly precipitated copper carbonate ($Cu(OH)_2.CuCO_3$), made by mixing equivalent amounts of copper sulphate and sodium carbonate. The third was grown in soil containing 0.05 per cent of copper in the form of finely pulverized chalcocite.

The samples were harvested with care to prevent contamination with copper dust; the root portions being washed in copper-free water saturated with carbon dioxide until the washings contained no trace of copper. Determinations of copper, as

[5] Blyth, Poisons, fourth edition, pp. 640–641.

usual, were made as shown under "Methods of Analysis" (see Appendix herewith). Following are the tabulated results:

TABLE VIII

ELEVEN STALKS OF CORN GROWN IN SOIL CONTAINING 0.01 PER CENT COPPER AS $Cu(OH)_2.CuCO_3$ (1907)

| No. | Plant part | Weight of sample, grams | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 3869*p* | Lower six nodes, 24 in. long | 43.4 | .00012 | 3.00 |
| 3869*q* | Basal sheaths of leaves from lower six nodes | 23.2 | .0001 | 4.00 |
| 3869*r* | Blades of leaves from lower six nodes | 33.1 | .00029 | 9.00 |
| 3869*s* | Upper four-seven nodes, 24 in. long | 24.2 | .00017 | 7.00 |
| 3869*t* | Basal sheaths of leaves from upper nodes | 20.6 | .00013 | 6.00 |
| 3869*u* | Blades of leaves from upper nodes | 19.6 | .00024 | 12.00 |
| 3869*v* | Rudimentary ears | 11.8 | .0001 | 9.00 |
| 3869 | Whole top portions | 175.9 | .00115 | 6.50 |
| 3868 | Roots | 10.6 | .00161 | 152.00 |

TABLE IX

TEN STALKS OF CORN GROWN IN SOIL CONTAINING 0.025 PER CENT COPPER AS $Cu(OH)_2.CuCO_3$ (1907)

| No. | Plant part | Weight of sample, grams | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 3865*a* | Five lower nodes, 14.4 in. long | 17 | .00024 | 14.00 |
| 3865*b* | Basal 12 in. of leaves and sheaths from five lower nodes | 17.2 | .00037 | 22.00 |
| 3865*c* | Terminal 14 in. of leaves from five lower nodes | 14.4 | .00047 | 33.00 |
| 3865*d* | Upper five-seven nodes, 14 in. long, including tassels and ears | 9.2 | .00017 | 19.00 |
| 3865*e* | Leaves from same | 19.2 | .00035 | 18.00 |
| 3865 | Whole top portions | 77 | .0016 | 21 |
| 3866 | Roots | 9.2 | .0067 | 728 |

TABLE X

FOUR STALKS OF CORN GROWN IN SOIL CONTAINING 0.05 PER CENT COPPER AS $Cu_2S$. (1908)

| No. | Plant part | Weight of sample, grams | Cu found, grams | Cu p.p.m. |
|---|---|---|---|---|
| 3968*p* | Lower six nodes | 11.5 | .00010 | 9.00 |
| 3968*q* | Basal sheaths from lower six nodes | 7.5 | .00008 | 11.00 |
| 3968*r* | Blades from do. | 15.7 | .00021 | 13.00 |
| 3968*s* | Upper five-six nodes | 3.5 | .00004 | 11.00 |
| 3968*t* | Basal sheaths from upper five-six nodes | 5.2 | .00007 | 13.00 |
| 3968*u* | Blades from do. | 4.9 | .00010 | 20.00 |
| 3968*v* | Rudimentary ears | 3.4 | .00005 | 15.00 |
| | Whole top portions | 51.7 | .00065 | 12.50 |
| 3978*a* | Fine roots | 3.23 | .00081 | 251.00 |
| 3978*b* | Coarse roots | 2.91 | .00024 | 83.00 |
| | whole root system | 6.14 | .00105 | 171.00 |

In all of the corn samples shown above, the copper content of root systems is very much greater than that in the top portions of the plants, amounting to twenty-three times, thirty-four times, and thirteen times as much, respectively. In the aerial parts of all samples copper increases slightly but uniformly towards the upper and outer portions of the plants. This must be an effect of transpiration, by which copper in solution is carried to the terminal portions of the plant and there deposited. The fine roots of one sample were found to contain about three times as much copper as the coarse roots—a fact which can be explained by the greater proportion of absorbing surface to weight in small roots.

With reference to toxic effects, the culture in 0.01 per cent copper carbonate showed only a faint yellow striping of leaves, with no checking of growth. The 0.025 per cent culture gave leaves which were strongly striped with yellow, and the total growth reduced to less than one-half. Toxic effects evident in the top portions of this culture are manifestly to be associated mainly with the greatly increased copper content of its roots, since total amounts of copper in the top portions remain small. The 0.05 per cent culture of copper in the form of $Cu_2S$, or finely powdered chalcocite, showed only faint toxic effects in the tops. The following summary indicates the relation between toxic effects and copper content of materials.

| Culture | Condition | Cu in tops p.p.m. | Cu in roots p.p.m. | Ratio |
|---|---|---|---|---|
| Copper carbonate, 0.01% Cu (precipitated) | Leaves faintly striped normal weight | 6.50 | 152.00 | 1:23 |
| Copper carbonate, 0.025% Cu (precipitated) | Leaves strongly striped three-fourths yellow, half weight | 21.00 | 728.00 | 1:34 |
| Copper sulphide, 0.05% Cu | Faintly striped leaves, normal weight | 12.50 | 171.00 | 1:13 |

In this table a general relation is shown between the toxic effects in the aerial portions of the plant, and the amounts of copper in root systems; but as to the soils employed toxic effects are influenced both by amounts and character of copper compounds present, as is shown further on following pages.

In view of the fact that the small increase of copper in the carbonate cultures, from 0.01 to 0.025 per cent, caused severe toxic effects attended by an increase of copper in root systems from 152 to 728 p.p.m. of dry matter, it seemed desirable to investigate thoroughly the quantitative relations between the copper in roots and the toxic effects as shown in vegetative growth. It was expected in this way to find a means of determining whether a plant contained an injurious or killing dose of copper, just as, analogously, killing doses of poisons in animals may be ascertained. With this end in view cultures of corn, beans, and squashes were grown in water, in pots of soil and in garden plots; and roots and top portions were examined quantitatively for copper.

In preparing samples of roots for analysis, washing with 4 per cent hydrochloric acid was carried out with water cultures, but most of the samples were prepared by washing with large quantities of copper-free water saturated with carbon dioxide, until the washings showed no trace of copper. By still a third method the soil adhering to a sample was analyzed for copper, the ash was then determined and assumed to be soil, and a corresponding amount of copper subtracted from the total found. For details see "Methods of Analysis." All of these methods undoubtedly give conservative figures for copper in root systems inasmuch as solvents not only remove externally adhering compounds but may also gradually act upon the copper content of

root systems. The acid-wash and soil-correction methods give severely minimum results. The carbon dioxide wash used in the majority of analyses is laborious but more satisfactory.

## WATER CULTURES (1907)

Cultures of corn, beans, and squash were grown in University of Arizona well-water containing 250 p.p.m. of soluble solids. From 0.03 to 3.0 parts of copper as precipitated carbonate dissolved in carbon dioxide were used in making cultures and the resulting growths of tops and roots were divided into the worst-poisoned and least-poisoned portions, for determinations of copper.

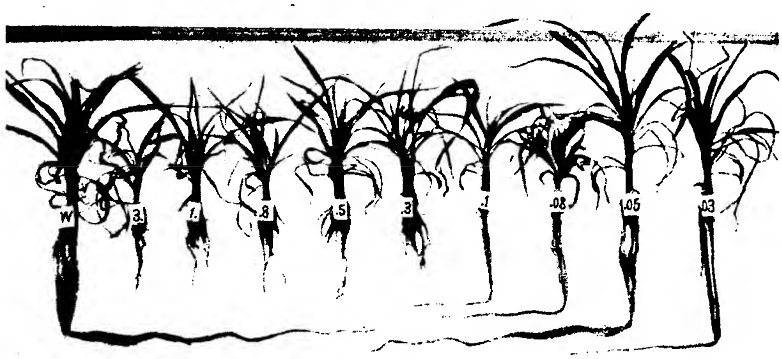


Fig. 3.—Corn cultures, series 121-62, grown in University of Arizona well water, containing from .03 to 3. parts per million of copper as basic carbonate ($Cu(OH)_2.CuCO_3$).

*Series Corn 121-62.*—Grown in well water containing Cu as $Cu(OH)_2.CuCO_3$ as follows: check, 3.0, 1.0, 0.8, 0.5, 0.3, 0.1, 0.08, 0.05, and 0.03 p.p.m. Cu. December 1-February 27, 1907. Series divided into two portions:

*a.* Plants not badly poisoned; roots growing; tops showing Cu effects; 0.1, 0.08, 0.05, 0.03 cultures. (Nos. 3694, 3693.)

*b.* Plants badly poisoned; root growth arrested; tops living; 3.0, 1.0, 0.8, 0.5, and 0.3 cultures. (Nos. 3692, 3691.)

*Series Beans 121-66.*—Grown in well water containing Cu as $Cu(OH)_2.CuCO_3$ as follows: check, 3.0, 1.0, 0.8, 0.5, 0.3, 0.1, 0.08, 0.05, 0.03 p.p.m. Cu. December 9-February 27, 1907. Series divided into two portions:

*a.* Least poisoned plants; roots nearly normal, tops normal; 0.3, 0.1, 0.08, 0.05, 0.03 cultures. (Nos. 3702, 3697.)

*b.* Worst poisoned plants; roots badly affected, tops less affected; 3.0, 1.0, 0.8, and 0.5 cultures. (Nos. 3700, 3699.)

*Series Squash 121–66.*—Grown in well water containing Cu as $Cu(OH)_2.CuCO_3$ as follows: check, 3.0, 1.0, 0.8, 0.5, 0.3, 0.1, 0.08, 0.05, and 0.03 p.p.m. Cu. December 10–February 27, 1907. Series divided into two portions:

*a.* Least poisoned plants; roots growing; tops strong; 0.3, 0.1, 0.08, 0.05, and 0.03 cultures. (Nos. 3698, 3701.)

*b.* Worst poisoned plants; roots dead or nearly so; tops badly affected; 3.0, 1.0, 0.8, and 0.5 cultures. (Nos. 3696, 3695.)

TABLE XI

COPPER CONTENT OF PLANTS IN WATER CULTURES

| No. | Series | Condition of sample | Dry matter in grams | Cu found, grams | Cu p.p.m. dry matter tops | Cu p.p.m. dry matter roots |
|---|---|---|---|---|---|---|
| 3694 | Corn, .1, .08, .05, .03 | Tops affected | 6.3 | .00009 | 14.30 | |
| 3693 | Corn, .1, .08, .05, .03 | Roots growing | 2.3 | .000236 | | 102.60 |
| 3692 | Corn, 3., 1., .8, .5, .3 | Tops living | 4.8 | .000056 | 11.70 | |
| 3691 | Corn, 3., 1., .8, .5, .3 | Roots arrested | 2.8 | .000572 | | 204.30 |
| 3702 | Beans, .3, .1, .08, .05, .03 | Tops normal | 9.4 | .000198 | 21.10 | |
| 3701 | Beans, .3, .1, .08, .05, .03 | Roots growing | 2.6 | .000157 | | 60.40 |
| 3700 | Beans, 3., 1., .8, .5 | Tops affected | 6.6 | .000204 | 30.90 | |
| 3699 | Beans, 3., 1., .8, .5 | Roots badly affected | 1.6 | .000494 | | 308.80 |
| 3698 | Squash, .3, .1, .08, .05, .03 | Tops strong | 10.4 | .000333 | 32.00 | |
| 3697 | Squash, .3, .1, .08, .05, .03 | Roots nearly normal | .6 | .000087 | | 145.00 |
| 3696 | Squash, 3., 1., .8, .5 | Tops badly affected | 3.6 | .000092 | 26.00 | |
| 3695 | Squash, 3., 1., .8, .5 | Roots dead | .2 | .000058 | | 290.00 |

It is noteworthy, in this series, that the amounts of copper found in roots that still retain the power of growth average about 103 parts in one million of dry matter, as compared with 268 parts in dead roots whose protoplasm is presumably killed as an effect of copper. Badly poisoned roots in every instance show a great excess of copper over those less affected. The tops, on the other hand, do not show copper in proportion to the amounts in the roots, averaging the same amount of copper in badly poisoned (22.9 p.p.m.) and in slightly poisoned (22.5 p.p.m.) plants. Corn was observed to be distinctly more sensitive to copper in water culture than either squash or beans, as was

shown by the method of measuring growth of root tips marked with India ink, and noting points at which growth was retarded (*R*) and arrested (*A*).

TABLE XII

SHOWING POINTS AT WHICH ROOTS WERE RETARDED OR ARRESTED IN GROWTH

| Cultures in Cu, in well water, parts per million | .03 | .05 | .08 | .1 | .3 | .5 | .8 | 1. | 3. |
|---|---|---|---|---|---|---|---|---|---|
| Corn | | R | | | | | A | | |
| Beans | | | | R | | | A | | |
| Squash | | | | R | | | A | | |

Photographs of the three series also indicate an earlier retardation of corn root development than of bean or squash root development; and show additionally that the top portions of cultures are not damaged in proportion to the root systems.

## TOXICITY OF COPPER SOLUTIONS TO PLANT ROOTS IN WATER CULTURE

In order to gain some indication of effects in water culture of copper salts upon plants, several series of plants were grown under varying conditions, and effects observed of the kind of copper salts employed, strength of solution used, the kind of plant, and the effects of other salts present.

Solutions were made in water free from copper, twice distilled; or, where permissible, University of Arizona well water, copper-free. The series were arranged, usually, to carry 0.01, 0.03, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8, 1.0, 3.0, and 5.0 parts copper per million of water. The cultures were made in 600-c.c. bottles, covered with pasteboard squares saturated with hot paraffin and perforated with three holes for plant seedlings held in place by cotton.

Effects upon cultures were judged by elongation of roots determined by the usual method of marking with India ink 5 mm. back of root tips and noting growth after twenty-four hours. Corn, beans, and squash were the plants employed and the points

particularly noted were those at which growth was retarded and at which it was arrested.

Table XIII gives the data condensed from the experimental records:

TABLE XIII (*a*)

TOXIC EFFECTS OF COPPER UPON ROOTS OF WATER CULTURES

First experiment (1905)

| Culture | Copper salt employed | Kind of water | Cu in solution: Growth retarded between, p.p.m. | Cu in solution: Growth arrested between, p.p.m. |
|---|---|---|---|---|
| Beans | $CuSO_4$ | Distilled | | .25—1.25 |
| Beans | $Cu(OH)_2.CuCO_3$ | Well | | .57—5.7 |
| Cantaloupes | $CuSO_4$ | Distilled | | less than .25 |
| Cantaloupes | $Cu(OH)_2.CuCO_3$ | Well | | .57—5.7 |

Indicating lessened toxicity in well water.

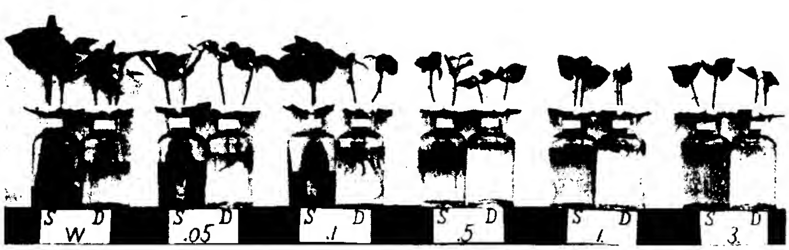


Fig. 4.—Bean cultures (eighth exp.), showing effects of varying concentrations of copper in distilled water and in solutions of mixed salts. *S*, salt solutions; *D*, distilled water; *W*, no copper, and .05 to 3. parts per million of copper.

TABLE XIII (*b*)

Eighth experiment (1905)

| | | | | |
|---|---|---|---|---|
| Corn | $Cu(OH)_2.CuCO_3$ | Salt solution* | .3 — .5 | .8 —1. |
| Corn | $Cu(OH)_2.CuCO_3$ | Distilled | less than .01 | .1 — .3 |
| Beans | $Cu(OH)_2.CuCO_3$ | Salt solution* | .1 — .5 | .8 —1. |
| Beans | $Cu(OH)_2.CuCO_3$ | Distilled | .1 — .3 | .5 — .8 |
| Squash | $Cu(OH)_2.CuCO_3$ | Salt solution* | .1 — .5 | .8 —1. |
| Squash | $Cu(OH)_2.CuCO_3$ | Distilled | .1 — .3 | .3 — .5 |

Showing lessened toxicity in salt solution.

| | |
|---|---|
| * NaCl | 64 pts. |
| $Na_2SO_4$ | 2 |
| $CaSO_4$ | 7.3 |
| Univ. well water salts | 26.1 |
| Total | 100 pts. per 100,000. |

TABLE XIII (*c*)

Fifth experiment (1905)

| | | | | |
|---|---|---|---|---|
| Corn | $CuSO_4$ | Distilled | .01— .05 | .1 — .5 |
| Corn | $Cu(OH)_2.CuCO_3$ | Distilled | .01— .05 | .1 — .5 |

Indicating equal toxicity of Cu as sulphate and as carbonate, and (compare second, fourth and eighth experiments) great toxicity to corn in distilled water.

TABLE XIII (*d*)

Third experiment (1905).

| | | | | |
|---|---|---|---|---|
| Beans | $CuSO_4$ | Distilled | | .1 — .3 |
| Beans | $Cu(OH)_2.CuCO_3$ | Distilled | | .1 — .3 |

Toxicity to beans of Cu as sulphate and as carbonate was the same.

TABLE XIII (*e*)

Seventh experiment (1905)

| | | | | |
|---|---|---|---|---|
| Squash | $CuSO_4$ | Distilled | .01— .05 | .1 — .5 |
| Squash | $Cu(OH)_2.CuCO_3$ | Distilled | .01— .05 | .1 — .5 |

Toxicity to squash of Cu as sulphate and as carbonate was the same.

TABLE XIII (*f*)

Second experiment (1905)

| Culture | Copper salt employed | Kind of water | Cu in solution: Growth retarded between, p.p.m. | Cu in solution: Growth arrested between, p.p.m. |
|---|---|---|---|---|
| Corn | $Cu(OH)_2.CuCO_3$ | Well | .1 — .3 | .8 —1. |
| Beans | $Cu(OH)_2.CuCO_3$ | Well | .1 — .3 | .8 —1. |

Toxicity of copper as $Cu(OH)_2.CuCO_3$ to corn and beans was the same.

TABLE XIII (*g*)

Fourth experiment (1905)

| | | | | |
|---|---|---|---|---|
| Corn | $Cu(OH)_2.CuCO_3$ | Well | .05— .08 | .8 —1. |
| Squash | $Cu(OH)_2.CuCO_3$ | Well | .1 — .3 | .8 —1. |

Corn was somewhat more sensitive to copper as $Cu(OH)_2.CuCO_3$ than squash.

TABLE XIII (*h*)

Sixth experiment (1905)

| | | | | |
|---|---|---|---|---|
| Beans | $Cu(OH)_2.CuCO_3$ | Well | .1 — .3 | 1. —3. |
| Squash | $Cu(OH)_2.CuCO_3$ | Well | .1 — .3 | .8 —1. |

Beans and squash were about equally sensitive to copper as $Cu(OH)_2.CuCO_3$

These experiments, which are not stated in complete detail here, indicate quite clearly:

1. That the toxic effects of copper are less in the presence of the salts ordinarily contained in well waters than in distilled-

water solution. This fact indicates that the toxicity of copper salts in the presence of soil-water solutions is probably minimized.

In all cases it was observed that root growth was much more vigorous in salty than in distilled water, where no copper was used. Lessened toxicity of copper in salty solutions may therefore *in part* be due to greater vigor and resistant qualities of plant cells grown in such solutions.

2. Copper appears to be equally toxic as sulphate or as basic carbonate.

3. Corn is probably more sensitive to copper salts than is squash or beans.

## Stimulation Effects in Water Cultures

In view of the debated question as to stimulation of plant growth by minute amounts of copper salts, it is of interest to observe that, quite consistently, the most vigorous root growth is associated with concentrations of from 0.01 to 0.1 parts per million of copper, as shown by details from cultures described on previous pages.

TABLE XIV (*a*)

Stimulation Effects of Copper upon Roots of Plants in Water Cultures

*Corn roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Condition |
|---|---|---|
| check | 23.4 mm. | Tops of plants showing increased growth at .08 and .1 p.p.m. |
| .01 | 27.3 | |
| .03 | 17.6 | |
| .05 | 17.3 | |
| .08 | 19.4 | |
| .1 | 18.5 | |

Showing stimulation at .01 p.p.m.

TABLE XIV (*b*)

*Bean roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Condition |
|---|---|---|
| check | 2.5 mm. | Tops of plants in .08 and .1 cultures higher than in .05, .03, .01, and check. |
| .01 | 2.2 | |
| .03 | 4.7 | |
| .05 | 2.8 | |
| .08 | 2.5 | |
| .1 | 2.9 | |

Showing stimulation at .03 p.p.m.

TABLE XIV (*c*)

*Corn roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Condition |
|---|---|---|
| check | 14.2 mm. | vigorous |
| .03 | 17.3 | most vigorous |
| .05 | 14.4 | most vigorous |
| .08 | 9.6 | retarded |
| .1 | 10.3 | retarded |

Showing stimulation at .03 p.p.m.

TABLE XIV (*d*)

*Squash roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m | Elongation 48 hrs. |
|---|---|---|---|
| check | 12.0 mm. | .08 | 12.3 |
| .03 | 10.7 | .1 | 13.2 |
| .05 | 10.0 | | |

Showing no stimulation at these concentrations.

TABLE XIV (*e*)

*Bean roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Condition |
|---|---|---|
| check | 2.4 mm. | Tops strong through- |
| .03 | 3.1 | out, showing stimula- |
| .05 | 4.5 | tion at .03, .05, and .1 |
| .08 | 2.8 | |
| .1 | 3. | |

Showing stimulation at .05 p.p.m.

TABLE XIV (*f*)

*Squash roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 13.1 mm. | .08 | 7.6 mm. |
| .03 | 7.4 | .1 | 9.7 |
| .05 | 8.8 | .3 | 3.7 |

Not showing stimulation consistently.

TABLE XIV (*g*)

*Corn roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 9.8 mm. | .1 | 13.5 mm. |
| .01 | 13.8 | .3 | 10. |
| .05 | 17.5 | .5 | 3.8 |

Showing strong stimulation .01 to .1 mm.

TABLE XIV (*h*)

*Corn roots* grown in distilled water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 27.6 mm. | .05 | 5.7 mm. |
| .01 | 1.8 | .1 | 2.7 |

No stimulation; eccentric results.

TABLE XIV (*i*)

*Bean roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 2. mm. | .1 | 6. mm. |
| .05 | 6. | .5 | .8 |

Showing stimulation at .05 to .1 p.p.m.

TABLE XIV (*j*)

*Bean roots* grown in distilled water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 3. mm. | .1 | 3.1 mm. |
| .01 | 3. | .3 | 1.6 |
| .05 | 3.7 | | |

Showing no stimulation.

TABLE XIV (*k*)

*Squash roots* grown in well water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 2.4 mm. | .1 | 5.7 mm. |
| .05 | 4.9 | .5 | .4 |

Showing stimulation at .05 to .1 p.p.m.

TABLE XIV (*l*)

*Squash roots* grown in distilled water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 3.3 mm. | .05 | 3.1 mm. |
| .01 | 3.3 | .1 | 2.1 |

Showing no stimulation.

TABLE XIV (*m*)

*Bean roots* grown in distilled water with $CuSO_4$

| Cu p.p.m. | Elongation 48 hrs. | Height of tops |
|---|---|---|
| .1 | 2.9 mm. | 87 mm. |
| .3 | 1.2 | 91 |
| .5 | .6 | 85 |

*Bean roots* grown in distilled water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Height of tops |
|---|---|---|
| .1 | 2.9 mm. | 98 mm. |
| .3 | 1. | 88 |
| .5 | .6 | 84 |

Showing same behavior with $CuSO_4$ and $Cu(OH)_2.CuCO_3$.

TABLE XIV (*n*)

*Squash roots* grown in distilled water with $CuSO_4$

| Cu p.p.m. | Elongation 24 hrs. | Cu p.p.m. | Elongation 24 hrs. |
|---|---|---|---|
| check | 3.6 mm. | .05 | 1.4 mm. |
| .01 | 2.8 | .1 | 2. |

*Squash roots* grown in distilled water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 24 hrs. | Cu p.p.m. | Elongation 24 hrs. |
|---|---|---|---|
| check | 3.6 mm. | .05 | 1.1 mm. |
| .01 | 3.8 | .1 | .4 |

Doubtful stimulation at .01 p.p.m.

TABLE XIV (*o*)

*Corn roots* grown in distilled water with $CuSO_4$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 8.7 mm. | .05 | 4.7 mm. |
| .01 | 10.9 | .1 | 1.5 |

*Corn roots* grown in distilled water with $Cu(OH)_2.CuCO_3$

| Cu p.p.m. | Elongation 48 hrs. | Cu p.p.m. | Elongation 48 hrs. |
|---|---|---|---|
| check | 8.7 mm. | .05 | 3.3 mm. |
| .01 | 13.2 | .1 | 2. |

These cultures, while somewhat fragmentary, afford excellent indications of stimulating effects upon plant roots. Excluding squash, which is not satisfactory material to work with, corn and beans show consistent stimulations at very high dilutions. Measurements in all cases are averages of about ten observations.

TABLE XV

SUMMARY OF STIMULATION EFFECTS

| Experiment | Culture | Copper salt used | Character and strength in copper of solution producing stimulation: Well water[8] | Distilled water |
|---|---|---|---|---|
| *a* | Corn roots | $Cu(OH)_2.CuCO_3$ | at .01 p.p.m. | |
| *b* | Bean roots | $Cu(OH)_2.CuCO_3$ | .03 | |
| *c* | Corn roots | $Cu(OH)_2.CuCO_3$ | .03 | |
| *e* | Bean roots | $Cu(OH)_2.CuCO_3$ | .05 | |
| *g* | Corn roots | $Cu(OH)_2.CuCO_3$ | .01–.1 | |
| *h* | Corn roots | $Cu(OH)_2.CuCO_3$ | | none at .01 or above |
| *i* | Bean roots | $Cu(OH)_2.CuCO_3$ | .05–.1 | |
| *j* | Bean roots | $Cu(OH)_2.CuCO_3$ | | none at .01 or above |
| *o* | Corn roots | $CuSO_4$ | | .01 |
| *o* | Corn roots | $Cu(OH)_2.CuCO_3$ | | .01 |

Only at very high dilutions (one part of copper to from 10,000,000 to 100,000,000 of water) are accelerations of root growth observed. These occur with both corn and beans, in well water. In distilled water stimulation was observed only at the highest dilution—1:100,000,000. In well water stimulation was observed at from 1:100,000,000 to 1:10,000,000—consistently with the well known fact that in presence of other soluble salts the effects of copper are lessened.

## EFFECTS OF SOIL UPON TOXICITY OF COPPER SOLUTIONS

Of prime importance in connection with possible toxic effects of copper in soils are the various reactions (1) converting insoluble into soluble compounds, (2) reconverting these again into insoluble combinations, and (3) modifying the toxic effects of copper salts in solution.

As shown in the table of solubilities, both basic carbonate of copper and chrysocolla are soluble in carbon dioxide, forming solutions which in water cultures are highly toxic in character. Sulphides of copper are first oxidized to the sulphate, which is easily soluble:

$$Cu_2S + 5O = CuSO_4 + CuO$$

For instance, 100 grams of chalcocite ore containing 3.2 per cent copper were shaken in a flask with 600 c.c. of water, frequently, during twenty-eight days. At the end of that time 500 c.c. of solution contained 0.0132 grams of copper.

Copper sulphate then reacts in the soil to form various insoluble compounds with consequent lessening of toxic action. With calcium carbonate the following represents a reaction which may occur:

$$2\,CuSO_4 + 2\,CaCO_3 + H_2O = Cu(OH)_2.CuCO_3 + 2\,CaSO_4 + CO_2$$

For instance, two grams of precipitated carbonate of lime were added to an excess of ten grams of copper sulphate in one liter

[6] "University of Arizona well water" contains 250 p.p.m. of soluble solids, mainly sodium sulphate.

of water, and digested with frequent shaking for over four months, the green precipitate being then filtered off, dried and analyzed for copper:

| | |
|---|---|
| Weight of precipitate taken | 100.00 mg. |
| Cu found | 47.85 |
| Theoretical Cu in basic carbonate | 57.38 |

Indicating by the formula above a conversion to basic carbonate of copper of over 83 per cent of the solid carbonate of lime present. Bicarbonate of lime in solution also reacts with copper sulphate to form the basic carbonate

$$CaH_2(CO_3)_2 + 4\,CuSO_4.5\,H_2O = 2\,Cu(OH)_2.CuCO_3 + CaSO_4 + 3\,H_2SO_4 + 16\,H_2O$$

$$3\,CaH_2(CO_3)_2 + 3\,H_2SO_4 = 3\,CaSO_4 + 6\,H_2O + 6\,CO_2$$

The silicates of the soil, also, and particularly those of zeolitic character, react readily with soluble copper compounds to form insoluble copper silicates. Organic matter likewise combines with large amounts of copper, to form compounds of indefinite or unknown composition. As a result of all these reactions, when soils are shaken up with solutions of copper salts the latter are withdrawn from solution in large amount. Under irrigation conditions, where waters containing minute amounts of copper are filtered through relatively large masses of soil, this action is nearly or quite complete.

Five large percolators were arranged with varying depths of

TABLE XVI

PERCOLATION OF COPPER SOLUTIONS THROUGH SOILS

| Soil | Depth | Solution used: Cu compound | Solution used: Cu in solution, p.p.m. | Amount of percolate, c.c. | Copper in percolate, p.p.m. |
|---|---|---|---|---|---|
| Sandy loam | 1 in. | $Cu(OH)_2.CuCO_3$ in $CO_2$ water | 95 | 2000 | none |
| Sandy loam | 5 in. | $Cu(OH)_2.CuCO_3$ in $CO_2$ water | 95 | 1500 | none |
| Sandy loam | 9 in. | $Cu(OH)_2.CuCO_3$ in $CO_2$ water | 95 | 2000 | none |
| Sandy loam | 1 in. | $Cu(OH)_2.CuCO_3$ in $CO_2$ water | 56 | 2000 | .85 |
| Heavy clay containing .003% Cu | 12 in. | $Cu(OH)_2.CuCO_3$ in $CO_2$ water | 8.5 | 600 | |
| Heavy clay containing .003% Cu | 12 in. | $CuSO_4.5\,H_2O$ | 254 | 150 | 7.3 |

soil resting on filter paper supported by a perforated porcelain plate. Two soils, heavy clay and sandy loam, were employed; and two copper solutions, sulphate and bicarbonate.

In nearly all cases copper as basic carbonate was entirely removed from solution in percolating through as little as a single inch of sandy loam. Although appreciable amounts of copper sulphate passed out of a soil, the latter in that case itself contained a very small percentage of copper. Inasmuch as soluble copper in irrigating waters must be present ordinarily as basic carbonate, its complete withdrawal by thin layers of soil is significant in connection with irrigated crops.

## Irrigation Experiments

A set of cultures was arranged to test the effects upon crop plants of solutions of basic copper carbonate so applied as to filter through the soil before reaching the plant roots. Six-inch

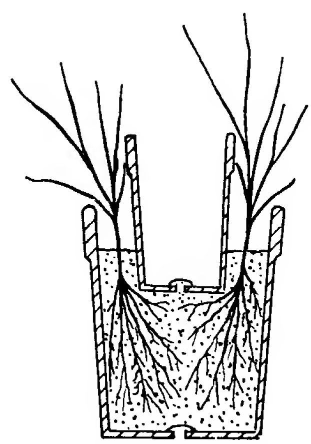

Fig. 5.—Diagram of pot culture irrigated through two-inch pot inside.

flower-pots were filled with sandy loam soil. In the middle of each of these pots a two-inch pot was half buried, and the plants experimented with were grown in the circles of soil between the large and small pots. These plants were irrigated by pouring the solution used into the small pot, through the bottom of which it passed, necessarily filtering through more or less soil before

reaching the plant roots. Radishes, beans, cantaloupes, cucumbers, lettuce, peas, beets, corn, berseem, avas, onions, barley, and wheat were employed; corn, barley, and wheat being especially successful under these conditions. All cultures were in pairs, one of each pair being irrigated with solutions of basic carbonate of copper in $CO_2$-water, and the check cultures with water only. In all other particulars—original strength of plants, exposure to light and air, and amount and time of watering—the conditions were identical.

These cultures were carried on in a greenhouse set aside for the purpose. The experiment was begun in November and ended the following March. The solutions of basic carbonate of copper employed contained from 0 to 55 p.p.m. of copper, averaging about 20 parts, which is from 7 to 670 times as much as has been observed in the waters of the Gila River from time to time.

TABLE XVII

CONDITION AT MATURITY OF CULTURES IRRIGATED WITH COPPER SOLUTIONS, AS COMPARED WITH THOSE IRRIGATED WITH WATER

C, copper culture; W, check.

| | | Tops | Roots |
|---|---|---|---|
| Radishes | | C and W. The same in appearance and weight | The same, but in C roots were removed $\frac{1}{3}$ in. from inner pot hole |
| Beans | C greener | C and W. About the same | Equal; same number of nodules; very local effect of Cu at pot hole |
| Lettuce | | C and W. About the same | The same except that in C roots were dead $\frac{3}{4} \times \frac{1}{3}$ in. under pot hole |
| Peas | | C and W. Averaging the same | Both C and W having abundant nodules. |
| Beets | | | No apparent damage by Cu |
| Corn | Stimulated? C showing stronger | Weighing the same, but C appearing stronger | Fewer in soil under pot hole in C, otherwise equal |
| Berseem | C stimulated, earlier bloom | C more advanced in growth, but not so heavy | Equally developed, both showing strong nodule development. |

| | | Tops | Roots |
|---|---|---|---|
| Avas | | C and W. Same apparent growth | In C roots within ¼ in. of pot hole damaged. Both C and W show strong nodule development |
| Onions | | C and W. Same general appearance | Very little local effect of Cu just under inner pot hole in C |
| Barley | C stimulated, matured over twice as much grain | The same in weight, but C matured more grain | No roots in C for space of 1 × ⅓ in. under inner pot hole |
| Wheat | C stimulated matured 20% more grain | Identical appearance, but C matured more grain | In C no roots under inner pot hole for space of 1¼ × ½ in. |

In practically all cases a distinct but very local effect of copper solutions upon plant roots under the inner pot hole was observed. For a distance of a half-inch or less from the small pot hole exposed roots were dead or missing. The soil in this area was observed in two instances to contain 0.25 and 0.45 per cent copper, respectively. In one instance 80 per cent of the copper added was found in the 43 grams of soil just under the bottom of the little pot, showing the rapidity with which copper is removed from its solutions by filtration through the soil.

The tops of the cultures under consideration in no instance showed injury, but in certain cases were in a distinctly advanced condition. The amounts of copper contained in material derived from these cultures are as follows:

TABLE XVII (*a*)

COPPER CONTENT OF PLANTS IRRIGATED WITH COPPER SOLUTIONS

| Sample No. | | Dry matter, grams | Cu grams | P.p.m. of Cu in dry material |
|---|---|---|---|---|
| 3673<br>3675 | Wheat and barley tops grown in check soil containing a trace (.0025 per cent) of copper | 32.90 | .000100 | 3.04 |
| 3672 | Tops of beans, peas, corn, lettuce, carrots, cucumbers and avas grown in check soil | 133.00 | .000350 | 2.60 |

| Sample No. | | Dry matter, grams | Cu grams | P.p.m. of Cu in dry material |
|---|---|---|---|---|
| 3674<br>3676 | Tops of wheat and barley irrigated with water averaging 20 p.p.m. copper | 30.70 | .000400 | 13.00 |
| | Tops of beans, berseem, peas, onions, lettuce, beets, radishes, corn, avas, barley, and wheat irrigated with water averaging 20 p.p.m. copper | 27.20 | .000751 | 27.60 |
| 3690 | Roots of same (washed in 4 per cent HCl) | 8.30 | .000750 | 90.00 |

*In brief,* even when relatively large amounts of water containing excessive quantities of soluble copper were applied and the experiments so arranged that all of the copper remained in the limited volumes of soil employed, no general injury to the plants was observed, although apparently slight stimulation occurred in some cases. Prolonged irrigation with such solutions would be required to saturate the soil to a depth sufficient to seriously injure plants grown in it.

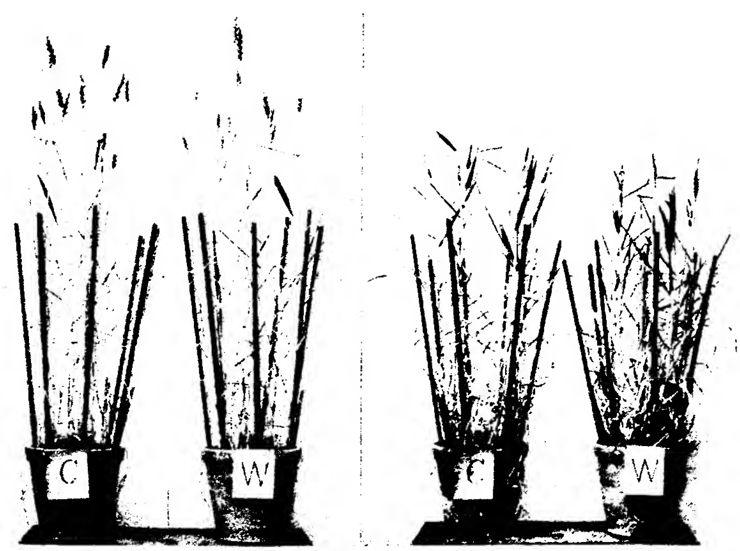


Fig. 6.—Wheat and barley irrigated (C) with copper solutions filtered through soil, and (W) with well water. Both show stimulated growth with copper.

## CULTURAL EXPERIMENTS

### Pot Cultures with Treated Soils

Pot cultures of corn, beans, and squash were also grown in soils containing copper in the form of precipitated carbonate ($Cu(OH)_2.CuCO_3$), finely powdered (100-mesh) chalcocite or sulphide ore, and finely powdered chrysocolla or silicate ore. Large glazed stone jars containing thirty-eight pounds of soil were used. Effects on growth were observed and the copper content of tops and of root systems was determined. The following tabulations relate to the work done in this direction. the statement showing the copper content of corn, bean, and squash plants expressed in parts per million of copper in dry matter.

TABLE XVIII

Copper Carbonate Series (1908), Beans

| Sample No. | Culture | Cu in soil, per cent | Appearance and height of plants | Dry matter, grams | Cu found, grams | Cu p.p.m. in tops | Cu p.p.m. in roots |
|---|---|---|---|---|---|---|---|
| | | | Normal | | | | |
| 3944 | Beans | Check* | 39 in. | 16.6 | .00022 | 13 | |
| 4013 | Beans | Check* | | .72 | .00033 | | 453 |
| 3945 | Beans | .01 | 38 | 17.2 | .00027 | 16 | |
| 4014 | Beans | .01 | | 1.35 | .00116 | | 859 |
| 3946 | Beans | .025 | 39 | 15.9 | .00033 | 21 | |
| 4015 | Beans | .025 | | 1.21 | .00115 | | 950 |
| Toxic effects begin at about .035% Cu in soil. | | | | | | | |
| | | | Stunted | | | | |
| 3947 | Beans | .05 | 30 | 13.2 | .00031 | 23 | |
| 4016 | Beans | .05 | | 1.09 | .00148 | | 1358 |
| 3948 | Beans | .1 | 25 | 6.7 | .00011 | 16 | |
| 4017 | Beans | .1 | | 1.44 | .00212 | | 1472 |
| 3949 | Beans | .25 | 14 | 3.7 | .00009 | 25 | |
| 4018 | Beans | .25 | | 1.35 | .00243 | | 1800 |
| 3950 | Beans | .5 | 15 | 2.9 | .0001 | 35 | |
| 4019 | Beans | | | .87 | .00147 | | 1690 |
| 3951 | Beans | 1. | 12 | 2.2 | .00009 | 41 | |
| 4020 | Beans | 1. | | .53 | .00106 | | 2000 |
| 3952 | Beans | 1.5 | 14 | 2.1 | .00009 | 44 | |
| 4021 | Beans | 1.5 | | .5 | .00115 | | 2300 |

* Containing traces of copper, .0025%.

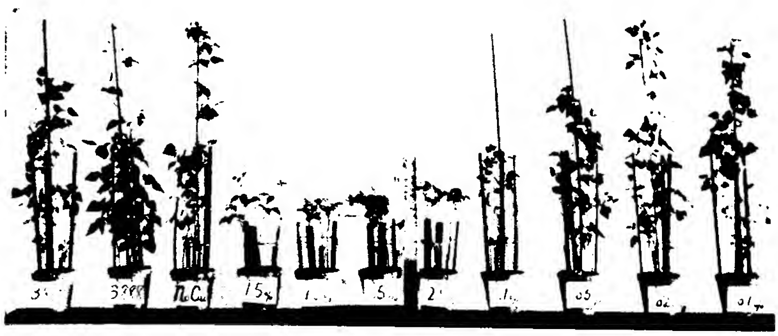

Fig. 7.—Bean cultures grown in soils containing copper as precipitated carbonate, from none to 1.5 per cent Cu.

TABLE XIX

COPPER CARBONATE SERIES (1907), CORN

| Sample No. | Culture | Cu in soil, per cent | Dry matter, grams | Cu found, grams | Cu p.p.m. in tops | Cu p.p.m. in roots |
|---|---|---|---|---|---|---|
| 3870 | Corn | Check* | 45.2 | .00020 | 4.40 | |
| 3885 | Corn | Check* | 8.8 | .00035 | | 40.00 |
| 3869 | Corn | .01 | 175.9 | .00115 | 6.50 | |
| 3868 | Corn | .01 | 10.6 | .00161 | | 152.00 |
| 3865 | Corn | .025 | 77.0 | .00160 | 21.00 | |
| 3866 | Corn | .025 | 9.2 | .00670 | | 728.00 |
| 3864 | Corn | .05 | 47.7 | .00103 | 22.00 | |
| 3867 | Corn | .05 | 4.4 | .00328 | | 745.00 |
| 3863 | Corn | .10 | 26.8 | .00079 | 30.00 | |
| 3862 | Corn | .15 | 9.8 | .00046 | 47.00 | |
| 3861 | Corn | .20 | 14.4 | .00073 | 51.00 | |
| 3860 | Corn | .30 | 4.6 | .00110 | 239.00 | |

* Containing traces of copper, .0025%.

Fig. 8.—Corn cultures grown in soils containing copper as precipitated carbonate, from none to .2 per cent Cu.

COPPER CARBONATE SERIES (1908), CORN

| Sample No. | Culture | Cu in soil, per cent | Appearance and height of plants | Dry matter, grams | Cu found, grams | Cu p.p.m. in roots |
|---|---|---|---|---|---|---|
| | | | Normal | | | |
| 3992 | Corn | Check* | 43 in. | 7.48 | .00058 | 78.00 |
| 3993 | Corn | .01 | 41 | 2.35 | .00049 | 209.00 |
| 3994 | Corn | .015 | 35 | 4.07 | .00171 | 420.00 |
| 3995 | Corn | .02 | 41 | 5.31 | .00397 | 748.00 |
| Toxic effects begin at about .023% Cu in soil. | | | | | | |
| | | | Stunted | | | |
| 3996 | Corn | .025 | 33 in. | 4.81 | .00245 | 509.00 |
| 3997 | Corn | .05 | 15 | .31 | .00023 | 742.00 |
| 3998 | Corn | .10 | 22 | 3.62 | .00651 | 1798.00 |
| 4000 | Corn | .20 | 20 | 1.99 | .00444 | 2231.00 |

* Containing traces of copper, .0025%.

COPPER CARBONATE SERIES (1908), SQUASH

| Sample No. | Culture | Cu in soil, per cent | Appearance and height of plants | Dry matter, grams | Cu found, grams | Cu p.p.m. in tops | Cu p.p.m. in roots |
|---|---|---|---|---|---|---|---|
| | | | Normal | | | | |
| 3937 | Squash | Check* | 16 in. | 11.2 | .00016 | 14.00 | |
| 3938 | Squash | .01 | 16 | 6.3 | .00023 | 36.00 | |
| 3939 | Squash | .025 | 15 | 9.2 | .00031 | 39.00 | |
| 4026 | Squash | Chk., .01, and .025 | | .24 | .00004 | | 169.00 |
| Toxic effects begin at about .035% Cu in soil. | | | | | | | |
| | | | Blanched and stunted | | | | |
| 3940 | Squash | .05 | 11 in. | 3.7 | .00017 | 46.00 | |
| 3941 | Squash | .10 | 11 | 2.3 | .00014 | 61.00 | |

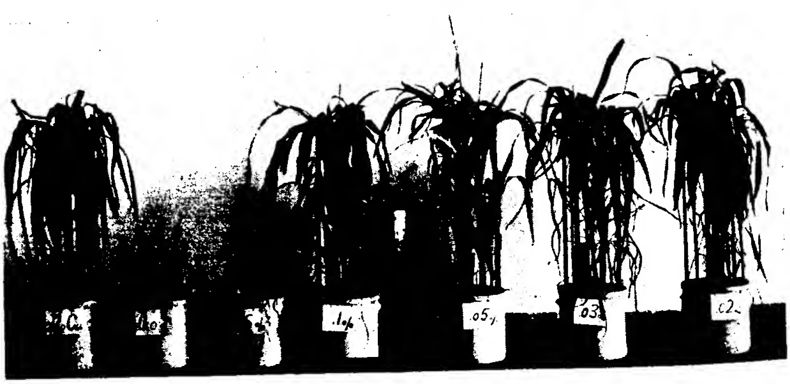

Fig. 9.—Corn cultures grown in soils containing copper as sulphide (chalcocite), from none to 1. per cent Cu.

TABLE XX

CHALCOCITE SERIES (1908)

| Sample No. | Culture | Cu in soil, per cent | Appearance and height of plants | Dry matter, grams | Cu found, grams | Cu p.p.m. in tops | Cu p.p.m. in roots |
|---|---|---|---|---|---|---|---|
| | | | Normal | | | | |
| 3979 | Corn | Check* | 36 in. | 17.60 | .00026 | 15.00 | |
| 3979*c* | Corn | Check* | | 4.62 | .00027 | | 58.00 |
| 3980 | Corn | .01 | 33 | 11.60 | .00011 | 10.00 | |
| 3980c | Corn | .01 | | 2.94 | .00023 | | 78.00 |
| 3981 | Corn | .02 | 38 | 19.40 | .00021 | 11.00 | |
| 3981c | Corn | .02 | | 6.14 | .00114 | | 186.00 |
| 3982 | Corn | .03 | 35 | 17.90 | .00028 | 16.00 | |
| 3982c | Corn | .03 | | 6.99 | .00176 | | 252.00 |
| 3968 | Corn | .05 | 45 | 51.70 | .00065 | 13.00 | |
| 3978 | Corn | .05 | | 6.14 | .00105 | | 171.00 |
| Toxic effects begin at about .08% Cu in soil. | | | | | | | |
| | | | Stunted | | | | |
| 3983 | Corn | .10 | 36 in. | 14.00 | .00031 | 22.00 | |
| 3983c | Corn | .10 | yellow | 6.08 | .00625 | | 1028.00 |
| 3984 | Corn | .50 | 8 in. | 3.20 | .00040 | 125.00 | |
| 3984c | Corn | .50 | | .47 | .00065 | | 1383.00 |
| 3985 | Corn | 1.00 | 12 | 3.20 | .00050 | 159.00 | |
| 3985c | Corn | 1.00 | | .49 | .00089 | | 1816.00 |

* Containing traces of copper, .0025%.

The cultures described in the foregoing tables indicate several interesting facts more or less applicable to field conditions.

(1) Precipitated carbonate of copper is shown to have a much more toxic effect upon corn than the finely pulverized ores of chalcocite or chrysocolla. With the precipitated carbonate 0.025 per cent in the soil was distinctly toxic, while with chalcocite and chrysocolla about 0.08 per cent was required to produce an equal effect. Inasmuch as all of these combinations of copper may occur in a soil subject to mining detritus, a mere determination of total copper in soils containing doubtfully toxic quantities cannot convey trustworthy information as to the injuriousness of the amounts present.

Moreover, since it has been shown that in the case of precipitated carbonate, and sulphate of copper, equivalent quantities of these salts in solution are equally toxic, it is probable that the greater toxicity of the carbonate is due to its greater solubility under soil conditions. It is, in fact, shown in table I, "Solubili-

TABLE XXI

CHRYSOCOLLA SERIES (1908)

| Sample No. | Culture | Cu in soil, per cent | Appearance and height of plants | Dry matter, grams | Cu found, grams | Cu p.p.m. in tops | Cu p.p.m. in roots |
|---|---|---|---|---|---|---|---|
| | | | Normal | | | | |
| 4003 | Corn | Check* | 32 in. | 25.60 | .00025 | 10.00 | |
| 4003c | Corn | Check* | | 6.46 | .00012 | | 19.00 |
| 4004 | Corn | .05 | 33 | 23.50 | .00026 | 11.00 | |
| 4004c | Corn | .05 | | 6.70 | .00062 | | 93.00 |
| Toxic effects begin at about .08% Cu in soil. | | | | | | | |
| | | | Dwarfed | | | | |
| 4005 | Corn | .10 | 30 in. | 17.90 | .00024 | 13.00 | |
| 4005c | Corn | .10 | striped | 5.82 | .00094 | | 162.00 |
| 4006 | Corn | .10 | 28 in. | 10.50 | .00017 | 16.00 | |
| 4006c | Corn | 1.00 | yellow | 4.29 | .00233 | | 543.00 |

* Containing traces of copper, .0025%.


Fig. 10.—Corn cultures grown in soils containing copper as silicate (chrysocolla), from none to 1. per cent Cu.

ties of Copper Compounds,'' that precipitated copper carbonate is soluble to the extent of 1.5 parts in 1,000,000 of water, while copper sulphide is soluble to the extent of 0.09 parts of copper in 1,000,000 of water. It is most probable, also, that the finely divided condition of the precipitated carbonate is more favorable to solution, and also to reaction with the acids of plant roots.

(2) Corn is seen to be distinctly more sensitive to the carbonate of copper than either beans or squash. With corn, toxic effects appear with 0.02 per cent of copper in the soil, while with beans and squash these toxic effects do not appear until 0.035 per cent of copper in the soil is reached. As is suggested

in the following pages, the physical constitution of root systems may account in part for varying degrees of sensitiveness to copper compounds.

The presence of copper in tops and roots of check is due to 0.0025 per cent of copper in the soil which was supposed originally to be free from this element.

### Pot Cultures with Field Soils

Two field soils containing copper from irrigating waters were tested in pot culture with reference to toxic effects and


Fig. 11.—Pot cultures of corn in field soils containing tailings. No. 3887, .027% Cu; no. 3888, .047% Cu; and no copper. Cultures in field soils are slightly affected.

copper content of root systems. The soils employed were from a field showing varying effects of accumulations of tailings, immediately southeast of Safford:

| | Sample | Cu in soil, per cent |
|---|---|---|
| 3887 | Sandy loam, surface 12 in. of soil recently put under irrigation | .027 |
| 3888 | Heavy clay (tailings) mixed with sandy loam, surface 12 in., long under irrigation, much tailings | .047 |

In these two soils, differing mainly through the addition of tailings to No. 3888, cultures of corn, beans, and squash were made, and examined for copper with the following results:

TABLE XXII

CULTURES IN TAILINGS SOILS

| No. | Pot culture | Condition | Cu in soil, per cent | Cu p.p.m. in tops | Cu p.p.m. in roots |
|---|---|---|---|---|---|
| 3887 | Corn in sandy loam | Distinctly striped | .027 | | 453.00 |
| 3888 | Corn in sandy loam and tailings | Less distinctly striped | .047 | | 163.00 |
| 3887 | Beans in sandy loam | Normal appearance | .027 | 28.00 | 1523.00 |
| 3888 | Beans in sandy loam and tailings | Normal appearance | .047 | 19.00 | 703.00 |
| 3887 | Squash in sandy loam | Yellow and stunted | .027 | 73.00 | |
| 3888 | Squash in sandy loam and tailings | Normal appearance | .047 | 45.00 | |

Fig. 12.—Showing effects of copper modified by tilth of soil. Strong growth, lumpy mixture; weak growth, thoroughly mixed.

Bean cultures appeared little affected by copper in either No. 3887 or No. 3888; but squash was distinctly damaged in No. 3887, being yellow and stunted. The leaves of both cultures of corn were paler than those of the check, but in soil No. 3887, containing less copper, the leaves of corn were more distinctly striped than in No. 3888. This is probably due to the sandy character of No. 3887 with consequently decreased adsorptive action upon copper salts. Lumpiness in the heavier soil might also account for a lessened toxic action, as indicated by an experiment in which 0.1 per cent of copper in the form of pre-

cipitated carbonate was mixed (1) intimately and (2) in lumpy condition. Results were as follows:

| Sample No. | Cu as pptd. carbonate, per cent | Condition | Cu p.p.m. in roots |
|---|---|---|---|
| 3998c | 0.1 well mixed | 22 in. high, much blanched | 1798.00 |
| 3999c | 0.1 lumpy | 28 in. high, mostly green | 457.00 |

In these instances it may be noted that toxic effects are associated with higher copper content of roots of plants, rather than with copper content of soils employed.

As in other cultures it is observed that beans, though carrying a higher copper content than corn, show less toxic effects—a fact possibly to be explained by the higher protein content of the plant with a consequently greater capacity for absorption of copper before toxic effects appear.

## Pot and Plot Cultures

In order to carry experimental cultures further towards field conditions, cultures of wheat and corn in small plots of sandy loam garden soil, 2½ × 18 feet, were grown, copper in the form of finely powdered sulphate having been thoroughly spaded in four times to a depth of nine inches in the amounts shown in table XXIII. The roots of these cultures were harvested and examined as usual for copper.

TABLE XXIII

Corn Grown in Garden Plots Containing Cu Applied as $CuSO_4$ (1914)

| Sample No. | Cu added, per cent | Condition of leaves | Dry matter, grams | Cu found, grams | Cu p.p.m. roots |
|---|---|---|---|---|---|
| 5858*a* | none | Solid green | 11.0 | .00015 | 14.00* |
| Toxic effects begin at about .008% Cu in soil. | | | | | |
| 5859*a* | .01 | Distinctly yellow striped | 11.6 | .00117 | 101.00 |
| 5860*a* | .025 | Distinctly yellow striped | 8.6 | .00211 | 246.00 |
| 5861*a* | .05 | Distinctly yellow striped | 7.3 | .00215 | 296.00 |
| 5862*a* | .10 | Strongly yellow striped | 4.3 | .00300 | 698.00 |
| 5863*a* | none | | 6.2 | .00013 | 21.00* |

* Probably resulting from roots spreading to copper soils.

## TABLE XXIV

WHEAT GROWN IN GARDEN PLOTS CONTAINING CU AS $CuSO_4$ (1914)

| Sample No. | Cu added, per cent | Condition of leaves | Dry matter, grams | Cu found, grams | Cu p.p.m. in roots |
|---|---|---|---|---|---|
| 5648*a* | none | 29 in. high; good | 4.46 | .00012 | 27.00 |
| 5649*a* | .01 | 29 in. high; good | 3.16 | .00190 | 601.00 |
| Toxic effects begin at about .02% Cu in soil. | | | | | |
| 5650*a* | .025 | 25-27 in. high; affected | 3.23 | .00260 | 805.00 |
| 5651*a* | .05 | 23 in. high; severely affected | 1.90 | .00330 | 1737.00 |
| 5652*a* | .10 | 20 in. high; very severely affected | 1.33 | .00200 | 1504.00 |

## TABLE XXV

WHEAT GROWN IN POTS TO CHECK PLOTS CONTAINING CU AS $CuSO_4$ (1914)

| Sample No. | Cu added, per cent | Condition of leaves | Dry matter, grams | Cu found, grams | Cu p.p.m. in roots |
|---|---|---|---|---|---|
| 5672*a* | .0025 | Green; 27 in. high | 1.51 | .00007 | 46.00 |
| Toxic effects begin at about .005% Cu in soil. | | | | | |
| 5673*a* | .01 | Yellowish; 23 in. high | 1.96 | .00035 | 179.00 |
| 5674*a* | .025 | Yellow and stunted; 17 in. high | .84 | .00030 | 357.00 |
| 5675*a* | .05 | Yellow and stunted; 12 in. high | .52 | .00031 | 593.00 |
| 5676*a* | .10 | Yellow and stunted; 4-12 in. high | .30 | .00044 | 1476.00 |

The corn series contains much smaller proportions of copper in the roots than either of the wheat series, a fact explained in part by the coarser roots of corn, which therefore have less absorptive surface in proportion to their weight. Wheat roots grown in plots show much more copper than pot samples, although the copper is much more toxic to the plants in pots than in plots, a contradiction not easily understood unless it be that other less favorable conditions of growth in pots were responsible for the backward condition of the plants.

### FIELD SAMPLES OF SOILS AND VEGETATION

In order to relate, if possible, the experimental work detailed on previous pages to samples of field material, roots of barley, wheat, oats and corn, were collected in the district studied and the amounts of copper in them determined. The samples of bar-

## TABLE XXVI

COPPER IN SOILS, AND IN ROOTS OF PLANTS GROWN IN FIELD SOILS CONTAINING MINING DETRITUS, NEAR SOLOMONVILLE AND SAFFORD (1914)

| Jan. 3, 1909 | | Dry matter, grams | Cu found, grams | Cu p.p.m. in tops | Cu p.p.m. in roots |
|---|---|---|---|---|---|
| 4008*a* | Barley tops selected for toxic effects from tailings soil (Wm. Gillespie), Solomonville, under Montezuma Canal | 13.90 | .00061 | 43.80 | |
| 4008*b* | Barley roots, ditto | 2.70 | .00160 | | 592.50 |
| 4009*a* | Oat tops selected for toxic effects from field one mile west of Solomonville, under Montezuma Canal | 28.30 | .00121 | 42.70 | |
| 4009*b* | Oat roots, ditto | 2.55 | .00025 | | 98.00 |

| March, 1914 | | Dry matter, grams | Cu found, grams | Cu p.p.m. yellow | Cu p.p.m. green | Per cent Cu in soils shaken from roots | |
|---|---|---|---|---|---|---|---|
| 5544*a* | Barley roots, yellow plants | 3.78 | .00037 | 98 | | .017 | |
| 5545*a* | Barley roots, green plants | 2.33 | .00290 | | 124 | | .006 |
| 5546*a* | Barley roots, yellow plants | 3.41 | lost | ...... | | | |
| 5547*a* | Barley roots, green plants | 3.80 | .00047 | | 123 | | |
| 5548*a* | Wheat roots, yellow plants | 1.40 | .00044 | 314 | | .054 | |
| 5549*a* | Wheat roots, green plants | 1.25 | .00048 | | 382 | | .014 |
| 5550*a* | Barley roots, less green plants | 2.17 | .00077 | 354 | | | |
| 5551*a* | Barley roots, stronger plants | 3.34 | .00110 | | 329 | | |
| 5552*a* | Oat roots, yellow plants | 1.67 | .00066 | 394 | | .050 | |
| 5553*a* | Oat roots, green plants | 1.77 | .00030 | | 169 | | .039 |
| 5554*a* | Barley roots, yellow plants | 1.38 | .00057 | 411 | | .073 | |
| 5555*a* | Barley roots, green plants | 1.42 | .00041 | | 289 | | .032 |
| | Average | | | 314 | 236 | .048 | .023 |
| 4010 | Corn roots (1908) in tailings soil, Solomonville | 16.12 | .00097 | | 60 | | |
| November, 1914 | | | | | | | |
| 5841*a* | Corn roots, tailings 9 in. deep | 10.18 | .00021 | | 21 | | |
| 5842*a* | Corn roots, tailings 8 in. deep | 11.08 | .00038 | | 34 | | |
| 5843*a* | Corn roots, old tailings | 10.01 | .00038 | | 38 | | .055 |
| 5844*a* | Corn roots, tailings 6–12 in. deep | 21.48 | .00039 | | 18 | | |
| 5845*a* | Corn roots, old tailings | 16.24 | .00068 | | 42 | | |
| 5846*a* | Corn roots, old tailings | 5.02 | .00034 | | 68 | | .105 |
| 5847*a* | Corn roots, old tailings | 14.42 | .00104 | | 72 | | .040 |
| | | | | Average | 42 | | |

ley, wheat and oats were collected in sets of two in a place. One of each set was green, healthy growth, the other more or less yellow and unthrifty in appearance. The object of this method of sampling in soils found to contain small amounts of copper was, if possible, to relate unthrifty appearance of plants examined to copper found in roots and surrounding soil. Table 26 (p. 441) contains the results of the determinations made.

As may be expected under field conditions, which are more complex and variable than those of plot or pot cultures, these data are considerably contradictory. Roots of yellow barley, wheat and oat plants, for instance, in 5544*a* and 5548*a* contain less copper than roots of strong green plants grown alongside; although the average copper content (314 parts) of yellow and more or less unthrifty plants is seen to be greater than in green plants alongside (236 parts). So far as observed, the larger percentages of copper found in soils shaken from roots of the plants are always associated with yellow plants. The average copper content of soils from roots of yellow plants is 0.048 per cent, while that from green plants is 0.023 per cent. These observations indicate that in a general way the larger amounts of copper found in these field soils are associated with larger amounts of copper in root systems and with yellow color in young plants. The percentages of copper observed in the soil, ranging up to 0.073 per cent in one instance, is surprisingly high, but toxic effects must be qualified by the character of the compounds, soluble salts in the soil, and other factors noted on preceding pages.

Yellowness of foliage also may be due to other causes than copper. Among these are: (1) too much water, as in low places: (2) alkali accumulations; (3) cold weather; (4) too much nitrogen in improper form, as in some old barnyards; (5) too little available nitrogen, as on new ground; (6) shade, and (7) insect pests and plant diseases. Malnutrition from any cause, in fact, usually expresses itself in the yellow or striped appearance of the leaves of these crop plants. Such appearance, therefore, cannot be attributed to copper present in the soil, without exclusion of other causes and sufficient confirmatory evidence.

As in the case of plot and pot cultures, corn roots are ob-

served to contain much less copper than other grain roots grown in similar soils, a fact to be attributed to the coarse character of field samples of corn roots.

## USE OF COPPER SULPHATE TO KILL MOSS IN IRRIGATING DITCHES

Clear irrigating water supplies, such as are derived from seepage and from wells, quickly become choked with mosses and algae in warm weather, entailing loss of water and expensive ditch cleaning. In order to test the application of copper to a running stream for the purpose of killing the growth of aquatic plants, an experiment was conducted, in October, 1906, upon the Flowing Wells ditch near Tucson, which at the time contained abundant aquatic growth.

A barrel of copper sulphate solution was prepared and placed at the head of the ditch. By means of a small outlet controlled by a stopcock, fifteen pounds per hour of $CuSO_4.5H_2O$ were added to the ditch flow, this amount being in the proportion of 1 part of copper to 100,000 of water. Most of the copper was immediately precipitated by the bicarbonate of lime present in the water; still more probably combined in insoluble form with the soil along the ditch; while the remainder acted with toxic effect upon the sensitive algae and the less sensitive mosses (*Potomogetons*) growing in the water. A short distance below the barrel, where algae and mosses, after twenty-five hours' exposure to copper, were brown and dead and breaking away from their points of attachment, .84 parts of copper in 1,000,000 of water remained in solution. Three miles below the barrel, where the mosses and algae were still plainly affected, traces only of dissolved copper were perceptible. A renewal of copper from point to point would therefore have been necessary in treating a long ditch by this method, which, however, proved too costly for adoption in the instance mentioned.[7]

It is of interest in this connection to note that in the early days of irrigation on the Gila River, mosses grew in such abundance in the clearer waters obtained from the river at that time,

[7] See Bibliography, p. 488, reference 32.

that considerable labor was required to keep the ditches clean. These mosses have now entirely disappeared from the upper canals, due in part to the turbid waters in which they will not grow, and in part, perhaps, to the dissolved copper from the mines.

## PHYSIOLOGICAL OBSERVATIONS ON TOXIC EFFECTS OF COPPER SALTS

### Quantitative Work

Citrus seedlings placed in copper sulphate solutions containing from 2.5 to 100 parts of copper in 1,000,000 of distilled water wilted in forty-eight hours, thus showing effects of toxicity. Root tips then all turned red with $K_4FeCy_6$. Red root-tips sectioned showed under low power red cells under bark and around center. Citrus, cucumber and bitter melilot roots grown in 10:1,000,000 copper solution all gave violet reaction with KOH, less delicate but more distinctive than $K_4FeCy_6$, since the purple biuret test indicates both copper and protein.

Cultures of wheat, peas, corn, beans, and other plants grown in soils containing from 0.005 to 0.1 per cent of copper in soil, gave only very doubtful root-tip reactions with $K_4FeCy_6$, although showing evident injury, especially in 0.1 per cent culture. There is an essential difference between water-culture roots *placed in* copper solutions and roots *grown in* soil. The first are killed by excess of copper salts contained; the second are yet living and growing resistantly in the soil.

A 0.1 per cent copper culture of corn, wheat, beans and cucumbers was washed out from the soil and gave superficial red coloration with $K_4FeCy_6$, but not internal. Living tissue is evidently inconsistent with sufficient amounts of copper to give a plain internal test. Therefore, the small amounts of copper known to be in poisoned but living root systems must be disseminated. It is, therefore, of interest to know the copper-protein ratio in poisoned but living root systems, such a ratio being more significant than the ratio of copper to the whole mass of root systems, which includes various proximate principles not concerned in copper fixation.

Two assembled samples of corn, radish, wheat, vetch and peas grown in soils containing 0.005 per cent and 0.05 per cent of copper were, therefore, very thoroughly washed out, copper determined, and nitrogen determined × 6¼ for protein. The amount of copper required to saturate vegetable protein was assumed at 11.7 per cent (14.655 per cent CuO)—the average of figures given in Mann's *Chemistry of the Proteids*, page 305.

(1) Roots grown in .005% Cu in soil ............ .4739000 gm.
Cu .0105% ............ .0000498
N. 2.26% = Protein 14.125% ............ .0669400
Cu required for saturation of protein
.06694 gm. × 11.7% = .007832 gm. Cu for saturation

$$\text{Per cent saturated} = \frac{.0000498}{.007832} = .637\%$$

(2) Roots grown in .05% Cu in soil ............ .3561000 gm.
Cu .0322% ............ .0001147
N. 2.76% = Protein 17.25% ............ .0614900
Cu required for saturation of protein
.06149 gm. × 11.7% = .007194 gm. Cu for saturation

$$\text{Per cent saturated} = \frac{.0001147}{.007194} = 1.594\%$$

| Summary: | Cu p.p.m. dry roots | Per cent saturation of protein with copper |
|---|---|---|
| (1) .005% Cu in soil ............ | 105 | 0.636 |
| (2) .05% Cu in soil ............ | 322 | 1.594 |
| Ratio (1) to (2) ............ | 3.07 | 2.51 |

*In brief,* 10 times as much copper in the soil resulted in 3 times as much copper in the entire root systems and 2.5 times as much in the protein of these root systems. This latter increase, however, is responsible for an increase in damage from almost nothing to very severe.

Further observations on the copper-protein saturation figure in roots grown in soil containing copper, were made on wheat and Canada peas, planted in pots containing soil mixed with varying percentages of copper in the form of precipitated basic carbonate. The pots contained 102 pounds of sandy loam, and were irrigated in a uniform manner from time to time as water was needed. Plantings were made January 3, 1916, and roots harvested May 15.

TABLE XXVII

OBSERVATIONS ON THE SATURATION WITH COPPER OF PROTEIN IN ROOTS GROWN IN SOIL TREATED WITH $Cu(OH)_2.CuCO_3$

| Lab. No. | Copper in soil, per cent | Material | Weight of sample, grams | Weight of Cu in sample, grams | Protein in sample, grams | Cu required to saturate protein, grams, factor 11.7% | Percentage of saturation of protein with Cu |
|---|---|---|---|---|---|---|---|
| 6396 | .005 | Wheat roots | 2.4726 | .00023 | .1069 | .0125 | 1.84 |
| 6397 | .02 | Wheat roots | 4.0122 | .00068 | .1660 | .0194 | 3.50 |
| 6398 | .06 | Wheat roots | 2.2236 | .00068 | .1231 | .0144 | 4.70 |
| 6399 | .10 | Wheat roots | 2.4658 | .00037 | .1401 | .0164 | 2.25 |
| 6401 | .005 | Canada pea roots | 2.6275 | .00085 | .3936 | .0461 | 1.85 |
| 6402 | .02 | Canada pea roots | 2.3844 | .00093 | .3684 | .0431 | 2.16 |
| 6403 | .06 | Canada pea roots | 2.0056 | .00053 | .2657 | .0311 | 1.70 |
| 6404 | .10 | Canada pea roots | 2.2708 | .00093 | .3747 | .0438 | 2.12 |

While the figures on saturation in the last column of the table vary without reference to the amount of copper in the soil and the degree of injury observed in the roots, yet they all show a very low ratio of copper found to copper required for saturation of protein present.

In both wheat and peas, injury was first shown at 0.02 per cent of copper in soil, increasing greatly with higher percentages. This injury, showing as a characteristic crinkly condition, is best seen in wheat and corn and has been observed in wheat roots grown in a soil containing as little as 0.017 per cent of copper.

A further quantitative study of copper effects on root systems was carried out in water culture with corn, wheat, and Canada peas. Paraffin (parowax) disks one-third of an inch thick and nine inches in diameter were employed, perforated with holes of suitable diameter by means of steel cork borers. These disks were supported on paraffin posts two and one-half inches high in four-quart deep graniteware pans containing the water culture solutions which were used. After soaking, the germinating seeds were planted in paraffin disks of suitable perforation and nutrient solution was then poured up to level of contact with seeds. At first tap-water was used; then a nutrient solution made up as follows:

| | |
|---|---|
| $KNO_3$ | 1.0 gm. |
| $MgSO_4$ | .05 |
| NaCl | .5 |
| $CaSO_4$ | .5 |
| $FeCl_3$ | .04 |
| Tap-water | 1.0 liter |

No phosphate was included because of its precipitating action on copper salts. After the cultures were about four weeks old they were changed to nutrient solutions containing small amounts of copper which was gradually increased from one to ten parts per million of solution. The solution was neutralized with normal $H_2SO_4$ (methyl orange indicator) to prevent precipitation of copper by dissolved carbonates. Following is a summary of the history of the cultures, each of which was increased to include several hundred plants:

WHEAT

| | |
|---|---|
| Dec. 16 | Planted in tap-water. |
| Dec. 24 | Transferred to nutrient solution, one-third strength. |
| Jan. 8 | Changed to nutrient solution, two-thirds strength. |
| Jan. 14 | To nutrient solution, full strength. |
| Jan. 17 | To nutrient solution containing 1 part Cu per million. |
| Jan. 21 | To nutrient solution containing 2 parts Cu per million. |
| Jan. 25 | To nutrient solution containing 6 parts Cu per million. |
| Jan. 28 | To nutrient solution containing 10 parts Cu per million. |
| Feb. 9 | Experiment terminated. |

A faint biuret test appeared after addition of 6 p.p.m. Cu. Also distinct $K_4FeCy_6$ test. Roots did not become flaccid, but the tops of cultures were dying back and prostrated markedly in comparison with roots of control culture.

CANADA PEAS

| | |
|---|---|
| Dec. 20 | Planted in tap-water. |
| Dec. 30 | Transferred to fresh tap-water. |
| Jan. 6 | Transferred to fresh tap-water. |
| Jan. 18 | Transferred to nutrient solution. |
| Jan. 20 | Changed to nutrient solution containing 1 part Cu per million. |
| Jan. 21 | To nutrient solution containing 2 parts Cu per million. |
| Jan. 25 | To nutrient solution containing 6 parts Cu per million. |
| Jan. 28 | To nutrient solution containing 10 parts Cu per million. |
| Feb. 6-8 | Experiment terminated. |

A faint biuret test appeared after addition of 6 p.p.m. Cu. Distinct $K_4FeCy_6$ test in root tips at end of experiment. Roots not flaccid, but plants distinctly affected and tops dying back more than those of control culture.

CORN

| | |
|---|---|
| Dec. 22 | Planted in tap-water. |
| Jan. 8 | Changed to nutrient solution, one-third strength. |
| Jan. 19 | To nutrient solution containing 1 part Cu per million. |
| Jan. 21 | To nutrient solution containing 2 parts Cu per million. |
| Jan. 25 | To nutrient solution containing 6 parts Cu per million. |
| Jan. 28 | To nutrient solution containing 10 parts Cu per million. |
| Feb. 9 | Experiment terminated. |

Giving distinct, faint biuret test after addition of 6 p.p.m. Cu; also $K_4FeCy_6$ test at end of experiment. Roots not flaccid at end of experiment, but tops of cultures about half dead, while tops of control culture were still in good condition.

These cultures, as shown by the notes, were exposed to copper solutions—wheat twenty-three days, peas eighteen days, corn twenty-one days. At the end of the experiment roots were not flaccid, but very faint biuret and distinct ferrocyanide tests were observed. In all cases top growth was affected, corn most, wheat next, and peas least. This material, as indicated above, is poisoned only just enough to show reactions in root tips, although tops are distinctly affected. It, therefore, represents minimum rather than maximum toxic conditions. Material was harvested and analyzed to show copper and nitrogen ratios; and by estimating the number of root tips in samples of corn, peas, and wheat the amount of copper per root tip, required to show faint tests, was found.

TABLE XXVIII

QUANTITATIVE DETERMINATIONS ON WATER CULTURES SHOWING SLIGHT TOXIC EFFECTS

CORN

| No. | Sample | Dry matter, grams | Cu found, grams | Cu p.p.m. in dry matter |
|---|---|---|---|---|
| 6321 | 265 tops | 17.695 | .00048 | 27.10 |
| 6320, 6323 | Coarse roots | 2.4277 | .00022 | 91.00 |
| 6319 | 1100 root tips | .77 | .00042 | 545.50 |

Amount of copper per root tip associated with slight toxic effects, $.00042 \div 1100 = .000000382$ gm.

PEAS

| No. | Sample | Dry matter, grams | Cu found, grams | Cu p.p.m. in dry matter |
|---|---|---|---|---|
| 6327 | 250 tops | 7.0850 | .00012 | 16.90 |
| 6325 | Coarse roots | 1.2827 | .00180 | 1400.00 |
| 6326 | Fine roots | .8393 | .00141 | 1680.00 |
| 6324 | 5500 root tips | .4462 | .00153 | 3428.00 |

Amount of copper per root tip required to show slight toxic effects, $.00153 \div 5500 = .000000278$ gm.

| | |
|---|---|
| Total roots examined for nitrogen | 4.41730 gms. |
| Albuminoids in roots (Alb. N. × 6¼) | .83929 |
| Copper required to saturate albuminoids (factor 11.7%) | .09819 |
| Total Cu found | .00784 |

Saturation $\frac{.00784}{.09819} = 7.99\%$

WHEAT

| No. | Sample | Dry matter, grams | Cu found, grams | Cu p.p.m. in dry matter |
|---|---|---|---|---|
| 6332 | 530 tops | 12.7 | .00165 | 129.90 |
| 6331 | Roots | .6902 | .00020 | 297.00 |
| 6330 | 16000 root tips | .3664 | .00103 | 2811.00 |

Amount of copper per root tip associated with slight toxic effects, $.00103 \div 16000 = .000000064$ gm.

| | |
|---|---|
| Total roots examined for nitrogen | 2.9223 gms. |
| Albuminoids in roots (Alb. N. × 6¼) | .30794 |
| Copper required to saturate albuminoids (factor 11.7%) | .03603 |
| Total Cu found | .001789 |

Saturation $\frac{.001789}{.03603} = 4.96\%$

These figures show, as usual, relatively small amounts of copper in tops of plants, with large amounts in roots, increasing from coarser to finer portions, until in the root tips corn contains 545, peas 3428, and wheat 2811 parts per million of copper in dry matter. For peas and wheat these are the largest proportions of copper thus far observed in any plant samples.

When the total amount of copper found in each sample is divided by the number of root tips employed, an extraordinarily small amount of copper is found necessary to bring about toxic effects. For instance

| | |
|---|---|
| One corn root tip (terminal 3 cm.) required.... | .000000382 gms. Cu |
| One pea root tip (terminal 1 cm.) required...... | .000000278 gms. Cu |
| One wheat root tip (terminal 1 cm.) required | .000000064 gms. Cu |

Moreover, the extent to which albuminoids in affected roots are saturated with copper—only 7.99 per cent for peas and 4.96 per cent for wheat—indicates a maximum effectiveness upon roots of small amounts of the metal.

## Reactions of Copper with Growing Points

Corn seedlings fifteen days old were fixed with cotton in tall 50-c.c. graduated Nessler tubes containing different strengths of copper sulphate in pure distilled water. The strengths of solution employed were 20, 10, 5, 2.5, and 1.25 p.p.m. There was a check culture with no copper. After three days, in all cases except the check, the roots were flaccid, showing contraction on graduations and giving biuret and ferrocyanide tests, increasing in strength from weaker to stronger concentration.

An experiment with pea seedlings gave similar results, but when the quantity of pea roots was increased and weak solutions, 2.5 and 1.25 p.p.m., were employed in small quantities (20 c.c.), the tests became much fainter.

Severed roots of corn, also, were observed to give as good tests as roots of living plants. A large number (seventy) of severed root tips placed in a small quantity (20 c.c.) of weak solution (5 p.p.m.) gave only a faint ferrocyanide test. These observations indicate that the concentration of copper in growing points is due to ionic dissociation and migration through the semi-permeable membranes of the root systems,[8] rather than to transpiration. The fainter test for copper in large quantities of root material indicates lessened toxicity of dilute solutions of copper in presence of excess of root materials.

Mature wheat, corn and pea plants in nutrient solutions, but not growing actively, were treated with gradually increasing amounts of copper from January 21 to February 2, as follows:

Wheat, Corn, and Pea Plants, Thirty-seven Days Old, Treated with Copper in Nutrient Solution

Jan. 21–25; nutrient sol. w. 2 p.p.m. Cu.
Jan. 25–28; nutrient sol. w. 4 p.p.m. Cu.
Jan. 28 to Feb. 5, nutrient sol. w. 10 p.p.m. Cu.

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[8] See Bibliography, p. 488, references 35, 36, 37, 38, 39, 40, 41, 42, 43, 44.

Feb. 5; very faint biuret test for copper, distinct ferrocyanide test.

Corn, forty-eight days old in 50 p.p.m. Cu sol., two days, gave faint biuret and ferrocyanide tests.

Corn, forty-eight days old in 500 p.p.m. Cu sol., two days, gave faint tests for copper.

From these observations it is evident that the nearly negative results shown are due either to nutrient salts present or to the older and therefore more quiescent material employed. To settle this question, the following experiments were made:

(1) Young (ten days) wheat and corn plants were placed in copper solution in distilled water and in nutrient solutions and observed after twenty and forty hours, as follows:

2.5 p.p.m. Cu, distilled water—20 hours

10 days old: Young wheat; flaccid?; strong biuret test; strong $K_4FeCy_6$ test
60 days old: Old wheat; not flaccid; no strong biuret test; distinct $K_4FeCy_6$ test
10 days old: Young corn; flaccid; strong biuret test; strong $K_4FeCy_6$ test
60 days old: Old corn; flaccid; no biuret test; old tips, faint $K_4FeCy_6$ test young tips, strong $K_4FeCy_6$ test

10 p.p.m. Cu, distilled water—20 hours

10 days old: Young wheat; flaccid?; strong biuret test; strong $K_4FeCy_6$ test
60 days old: Old wheat; not flaccid; faint biuret test; distinct $K_4FeCy_6$ test
10 days old: Young corn; flaccid; strong biuret test; strong $K_4FeCy_6$ test
60 days old: Old corn; flaccid?; distinct biuret test; strong $K_4FeCy_6$ test

40 p.p.m. Cu, distilled water—20 hours

10 days old: Young wheat; flaccid; strong biuret test; strong $K_4FeCy_6$ test
60 days old: Old wheat; flaccid?; faint biuret test; distinct $K_4FeCy_6$ test
10 days old: Young corn; flaccid; strong biuret test; very strong $K_4FeCy_6$ test
60 days old: Old corn; flaccid; strong biuret test; strong $K_4FeCy_6$ test

The above results indicate that old roots of corn and wheat are more resistant to the penetration of copper than are the young roots. This is shown by less flaccidity in the weaker solutions and by the fainter tests observed. A second series with greater strengths (5, 20, and 100 p.p.m.) and longer exposure (forty-five hours) showed distinctly less differentiation than in the case of the series above given in detail. This is to be expected, inasmuch as stronger solutions must overcome resistance of roots exposed to them more quickly, and the longer time employed would likewise tend to overcome differences existing in the first few hours of the experiment.

(2) Young and old wheat and corn roots were placed in 10 p.p.m. Cu in distilled water and 10 p.p.m. Cu in nutrient solution, with the following results:

10 p.p.m. Cu, distilled water—20 hours

10 days old: Young wheat; flaccid?; strong biuret test; strong $K_4FeCy_6$ test
60 days old: Old wheat; not flaccid; faint biuret test; distinct $K_4FeCy_6$ test
10 days old: Young corn; flaccid; strong biuret test; strong $K_4FeCy_6$ test
60 days old: Old corn; flaccid; distinct biuret test; strong $K_4FeCy_6$ test

10 p.p.m. Cu, neutralized nutrient solution—20 hours

10 days old: Young wheat; not flaccid; doubtful biuret test; faint $K_4FeCy_6$ test
60 days old: Old wheat; not flaccid; none or doubtful biuret test; faint $K_4FeCy_6$ test
10 days old: Young corn; not flaccid; faint biuret test; distinct $K_4FeCy_6$ test
60 days old: Old corn; not flaccid; distinct biuret test; distinct $K_4FeCy_6$ test

This shows very distinctly the prevention of toxic action upon plant roots through the protective action of other solids in solution, as already observed in water cultures by measurements of root growth. It is noteworthy in this connection that corn roots generally seem to be more sensitive to the action of copper salts than the roots of wheat or peas.

In order to examine still further into the relative resistance of old and young root systems to copper salts, a solution of 5 p.p.m. Cu in distilled water was used, the time being varied from twenty to two hundred hours. The results of these observations indicate that, with wheat and corn roots, the penetration of copper is distinctly more rapid in young than in old material. Peas did not give clear results.

It appears from these observations, first, that the accumulation of copper in plant roots is distinctly due to the migration of dissociated ions into the root systems, where they are fixed by protoplasm, in which combination they are identified by means of the biuret test. Second, the presence of nutrient salts very distinctly lessens the effect of a 10 p.p.m. copper solution upon sensitive young growing plant roots. Third, old quiescent plant roots developed in a nutrient solution are distinctly less sensitive to copper salts than young roots which are still actively growing.

The slow development of biuret tests for copper in such material after sufficient exposure to copper solutions, indicates the presence of protoplasm.

It is possible that the same observations may apply to other poisons, metallic or otherwise, brought into contact with absorp-

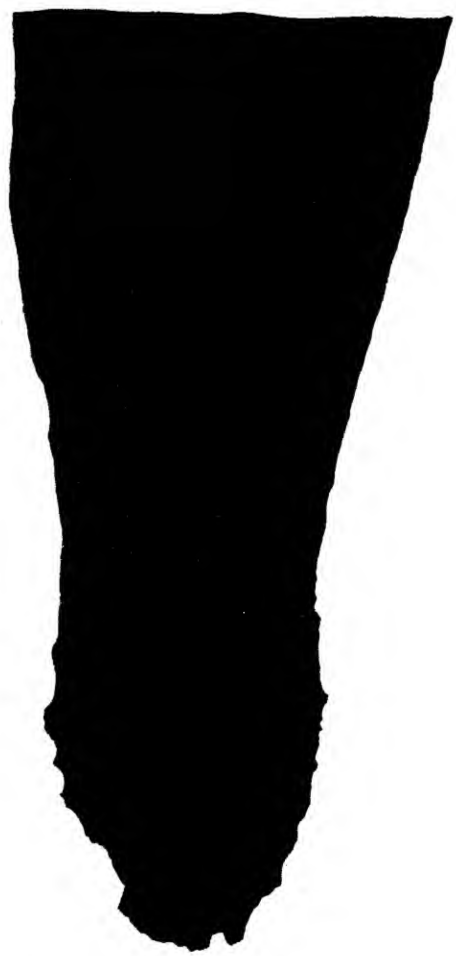


Fig. 13.—Photomicrograph of root tip of corn grown in water culture and poisoned by 1:200,000 of Cu in solution. The copper is shown as red copper ferrocyanide, which appears black in the photomicrograph. The irregular inner black line shows the penetration of the copper and also indicates sharply the differences in permeability of adjacent cells, some of which are penetrated before others. (× 80 diam.) (Photo by J. T. Barrett.)

tive root systems in the soil. Not only this, but it may be true that nutrient salts, as well, will be found more actively absorbed by younger and more sensitive root systems than by older ones, or by root systems which for any reason have become quiescent. This would suggest the possibility of choosing to advantage the proper time for applying substances, either to avoid injury or, as in the case of fertilizers, to secure maximum benefit from them.

### Varying Resistance of Individual Cells to Copper

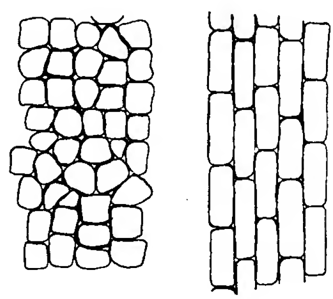
Not only do old and young roots vary as to toxic effects upon them of copper, but different degrees of resistance between individual cells in the same root and even in the same chain of cells, is clearly shown in the photomicrograph (fig. 13) of a corn root tip which has been exposed to a 1 to 200,000 solution of copper, then colored with $K_4FeCy_6$, and sectioned for observation. The dark, abruptly angular line of penetration shown in the section plainly indicates that individual cells may be penetrated by copper while adjacent cells growing under precisely similar physical conditions are not penetrated. If this be not due in some unseen way to morphological peculiarities of root structure, it must be due to individuality in the cells themselves, some of which must be more resistant to penetration by dilute copper solutions than others.

*Summing up the physiological observations relating to effects of copper upon plants*, we find (1) that individual cells vary (probably) in degree of resistance to penetration by copper salts; (2) that young roots are less resistant than old roots; (3) that roots of certain species of plants (e.g. corn) are less resistant than roots of other species; and (4) that toxic effects may be to some extent related to the structure and distribution of root systems.

## DIAGNOSIS OF COPPER INJURY

In the presence of toxic amounts of copper in the soil, the root systems of culture plants become harsh and crinkly with almost entire loss of root hairs. Consistent with the checking of growing points, root systems are also greatly restricted in extent,

and in feeding capacity. Individual roots are coarse, covered with thick epidermis, and are abruptly angular, apparently as a result of chemotropic contortions. Root tips are shortened and thickened and in some instances are strongly proliferated. The anatomical structures associated with these changes in form are very striking. In corn the cells of the primary cortex, in normal roots, are elongated parallel with the axis of the root, and in longitudinal tangential section measured about 74 by 30 microns. Injured cells of corn grown in soil containing 0.1 per cent copper gave longitudinal tangential sections approximately 34 by 30 microns, as shown on accompanying drawings. (See, also, plates 6, 7, and 8.



*a* *b*

Fig. 14.—*a*. Tangential longitudinal section of corn root grown in soil containing .1 per cent of copper as copper sulphate, showing cells of cortex of injured rootlet. *b*. Tangential longitudinal section of normal corn root cells of cortex. (× ± 300 diam.) (Sections by G. F. Freeman.)

These structural modifications, taken in connection with other symptoms and conditions and in the absence of other causes, such as an excess of alkali salts,[9] confirm a diagnosis for copper injury in a soil of doubtful toxicity. For instance, March 4, 1916, two sets of samples of barley were collected in the district studied, and the material examined for evidence of copper injury, as follows:

*Lot 1.*—Young barley plants from the upper end of a field midway between Safford and Solomonville, under Montezuma Canal. The soil next the ditch shows old tailings, and there are irregular areas of yellow barley immediately under the canal.

[9] See Livingston, Botanical Gazette, vol. 30, no. 5, p. 229, 1900.

| Sample No. | *a.* Yellow barley plants | |
|---|---|---|
| 6343 | Roots, crinkly and angular, much branched near surface. Dry weight, 3.2429 gms; Cu, .00085 gm; p.p.m. | 262 |
| 6344 | Soil shaken from yellow barley roots | |
| | Copper | .07979% |
| | Total soluble solids (alkali) | .46400 |
| | Cl as NaCl | .004 |
| | Sodium carbonate | .008 |
| | Nitrogen | .137 |
| | *b.* Green barley plants from near (*a*). | |
| 6345 | Roots, smooth and straight, not much branched near surface. Dry weight, 1.6025 gm; Cu, .0002 gm; p.p.m. | 125 |
| 6346 | Soil shaken from green barley roots | |
| | Copper | .05844% |
| | Total soluble solids (alkali) | .45600 |
| | Cl as NaCl | .004 |
| | Sodium carbonate | none |
| | Nitrogen | .181 |

*Lot 2.*—Young barley plants from the upper end of a field in West Layton under Montezuma Canal. Soil near ditch known to contain tailings and showing spots of yellow barley at head of field.

| Sample No. | *a.* Yellow barley plants | |
|---|---|---|
| 6347 | Roots, crinkly and angular, much branched. Dry weight, 3.2977 gms; Cu, .0014 gm; p.p.m. | 425 |
| 6348 | Soil shaken from roots of yellow barley plants | |
| | Copper | .1113% |
| | Total soluble solids (alkali) | .50 |
| | Cl as NaCl | .008 |
| | Sodium carbonate | .008 |
| | Nitrogen | .165 |
| | *b.* Green barley plants from near (*a*) | |
| 6349 | Roots, smooth, straight, not much branched. Dry weight, 2.2473 gms; Cu, .0003 gm; p.p.m. | 133 |
| 6350 | Soil shaken from roots of green barley plants | |
| | Copper | .02678% |
| | Total soluble solids (alkali) | .40 |
| | Cl as NaCl | .008 |
| | Sodium carbonate | .004 |
| | Nitrogen | .127 |

Considering the above observations, we notice that the soils from which samples were taken do not contain injurious amounts of soluble salts. Their nitrogen content, also, is normal. The areas of yellow barley from which samples come are therefore not to be attributed to alkali salts, or to abnormal nitrogen content. Observation in the field, also, failed to indicate that conditions of irrigation, temperature, or light were unfavorable, these conditions being the same for both green and yellow samples.

Excluding these considerations, therefore, we now find that there is uniformly more copper in the roots of yellow barley plants than in those of the green ones, also in the soils in which they occur. The roots of yellow plants, moreover, show the crinkly condition caused (though not exclusively) by copper when present in toxic amounts in the soil. The following statement summarizes these observations.

| | Cu in soil per cent | Cu p.p.m. in roots | Condition of roots |
|---|---|---|---|
| | | LOT 1 | |
| Yellow barley | .0798 | 262.00 | Crinkly and branched |
| Green barley | .0584 | 125.00 | Straight, not branched |
| | | LOT 2 | |
| Yellow barley | .1113 | 425.00 | Crinkly and branched |
| Green barley | .0268 | 133.00 | Straight, not branched |

The evidence therefore indicates quite conclusively that the two yellow samples owed their color to toxic effects of copper upon the roots of the young plants. Later in the season, however, no difference in mature plants, showing variations in color when young, may be observed. This must be due to the fact that as root systems penetrate more deeply into the soil they escape the surface zone of tailings, with consequent recovery from the effects of copper.

# Part II.—GENERAL DISCUSSION

## PRELIMINARY STATEMENT

The copper compounds, in solid form and in solution, that result from mining operations in the Clifton-Morenci district, have found their way down the San Francisco and Gila rivers to the underlying irrigated agricultural soils of Graham County in sufficient amounts to raise the question of their toxicity to crops. The largest amounts of copper in these soils are found at the heads of irrigated lands, especially where alfalfa is or has been, at which points old accumulations of tailings, laid down for the most part prior to 1908, are still to be found.

### Accumulations of Copper

The amounts of copper accumulating in the Gila River valley soils in this way are small, the observed range being from 0.006 per cent to 0.111 per cent in surface soils and the average for eighteen soils analyzed being 0.046 per cent of copper. Irrigated soils elsewhere have been observed to contain larger quantities of copper than those above noted, for instance 1.002 per cent on the Deer Lodge River below Anaconda, Montana, with an average of 0.09 per cent for eleven other samples taken in the same locality.[10]

These amounts of copper in a soil may or may not be toxic according to the combination in which the copper exists, the physical character of the soil and its chemical composition, climatic and moisture conditions, the crop grown, and other considerations which may now be discussed in order.

The small amounts of soluble copper constantly coming down stream from the mines which cannot, like solid tailings, be entirely excluded from irrigating water supplies, are of importance because of their tendency to accumulate by reason of the fixing power for copper of silicates, carbonates and organic matter in

salts were percolated through one to twelve inches of soils, with little or no copper appearing in the filtrates. Under field conditions, therefore, this action tends to concentrate dissolved copper in irrigating water in the surface few inches of the soil.

A series of samples of Montezuma Canal water taken at Solomonville affords quantitative suggestions in this connection:

TABLE XXIX

COPPER CONTENT OF GILA RIVER WATERS

| Sample No. and date | | Description | Amounts of Cu added in irrigation, estimated in p.p.m. of water | | | Approx. flow of Gila River in sec. ft. | Approx. amts. of copper carried down stream, 1 day lb. |
|---|---|---|---|---|---|---|---|
| | | | In tailings | In solution | Total | | |
| 3309 | May 26, '04 | River very low | 18.3 | .80 | 19.1 | 30 | 3094 |
| 3486 | June 11, '05 | Small flood | ...... | .25 | | 170 | 230 (Soluble only) |
| 3622 | June 25, '06 | River low | 1.6 | .11 | 1.71 | | |
| 3737 | Feb. 22, '07 | Medium flood | trace | 2.88 | ±3.0 | 600 | 9720 |
| 4011 | Jan. 3, '08 | | 2.1 | .08 | 2.18 | | |
| | | Tailings shut out of river May 1, 1908 | | | | | |
| 4029 | Apr. 12, '09 | | 1.4 | .08 | 1.48 | | |
| 6342 | Mar. 4, '16* | | .04 | .03 | .07 | | |

* Following four-months shut-down of operations in Clifton-Morenci district.

These figures, while somewhat meagre, seem to indicate a lessening waste of copper downstream following the restraint of tailings from the water-supply in May, 1908. This is especially true of copper in solution, due probably to the decreased amounts of solid copper compounds in suspension from which copper in solution is derived.

Assuming at the present time an average of 1 part of copper in 1,000,000 of Gila River water, four acre-feet of such water, required for one year's irrigation, would contain 10.9 pounds of copper, from which should be deducted small losses due to vegetation, drainage waters, and percolation to depths below the surface soil.

Six tons of alfalfa with a copper content of 5 p.p.m. contain 0.06 lb. copper; while one acre-foot of seepage water (about the annual seepage loss) containing 0.25 p.p.m. copper would carry 0.68 lb. copper. Estimating the total loss roughly at one pound

of copper per acre a year, the net addition of copper to the soil would be approximately ten pounds, or about 0.00025 per cent. It would therefore require about forty years to accumulate 0.01 per cent of copper in the surface foot of soil. Inasmuch as, under field conditions, this is not an injurious amount, there is little likelihood, considering the district in a general way, that the small residues of copper now coming down stream will accumulate to an injurious extent within a reasonable period of time. Incidentally, it is of interest to note the large total losses of copper (3094 lb. and 9720 lb. per day observed) formerly resulting from mining operations in the district.

## Possible Effects upon Health

With reference to the question of poisonous effects upon man and animals of dissolved copper in irrigating and well-waters, such effects, in general, are much less upon animals than upon plant life. Moore and Kellerman state, for instance, that 0.02 gms. of copper may be absorbed daily by a man with safety.[11] This amount of copper would be contained in five gallons of water containing one part per million of copper, the largest amount of copper observed in a well-water in the district studied being 0.53 p.p.m. It is of interest in this connection to note a belief of the copper miners of the Rio Tinto in southern Spain, where the wells are impregnated with copper, that one part of copper per million of drinking water is permissible, but that two parts per million result in "copper colic." [11a] In view of experiments upon human subjects, however, it is more than likely that deleterious effects observed are due to associated compounds in the water. It is of importance to note that a strength of as little as one part per million of copper in pure water will destroy algae, which are common in clear water supplies freely exposed to light and air. This fact may be made of use in cleaning ditches and reservoirs of aquatic growth, where the expense is not too great.

The germicidal effects of small amounts of copper in waters of the district studied also have a bearing upon human health.

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[11] U. S. D. A. Bur. Pl. Ind., Bull. 64, p. 23.

[11a] Conversation of J. W. Bennie, Clifton, Arizona.

Bacilli of various species reacting upon human health are very sensitive to the action of soluble copper salts. For instance, in distilled water "one part copper in 16,000,000 parts water killed typhoid bacilli in two hours. In copper solutions made up with tap and sea water, the action was still marked, but less vigorous than in distilled water."[12] Moore and Kellerman state that one part of copper sulphate to 100,000 parts of water destroys typhoid and cholera germs in three to four hours.[13] In milk supplies as little as one part of copper salts in 2,000,000 of water acts as an antiseptic against putrescent bacteria.[14] It seems, therefore, that there is a possibility that the amounts of copper observed in ditch and well-waters in the district may have an antiseptic effect upon malignant germs, more particularly typhoid fever, likely to occur in the district.[15]

## Amounts and Significance of Copper in Aerial Vegetation

The amounts of copper found in aerial parts of vegetation within the district are small, ranging from a trace to 7.6 parts copper in 1,000,000 of dry matter and averaging 3.41 parts. Miscellaneous cultures in water, potted soils, and plots gave larger amounts of copper which, however, were associated in most cases with toxic effects. Table 30 (p. 462) contains a summary of these data.

Even allowing for errors of method and of analysis, the European figures (3) seem excessively high, although the woody character of most of the samples was for the most part very different from that of the tender crop plants of the Arizona series.

Little can be said as to the toxic effects of the copper observed in aerial plant parts in the Arizona samples. The yellow striping of copper-poisoned corn is probably a general symptom of malnutrition to be attributed to the effect of copper upon root systems rather than upon leaves and stems. In rare instances, however, beans and squash in water culture showed dark green

[12] Biochem. Jour., Aug., 1908, pp. 319–323.

[13] U. S. D. A. Bur. Pl. Ind., Bull. 64, p. 43.

[14] Jour. Ind. and Eng. Chem., Sept., 1909, p. 676.

[15] See Bibliography, p. 487, references 3, 20, 21, 22.

TABLE XXX

SUMMARY OF COPPER CONTENT OF AERIAL VEGETATION

| | No. of samples | Min. | Max. | Ave. |
|---|---|---|---|---|
| | | Parts per million copper | | |
| 1. Field vegetation from upper Gila | 10 | trace | 7.60 | 3.41 |
| Field vegetation from other sources in Arizona | 9 | none | 6.30 | 1.52 |
| 2. Corn plants grown in pots .01–.05 per cent Cu | 3 | 6.5 | 21.00 | 13.30 |
| Tops of corn, beans and squash grown in Cu water culture | 6 | 11.7 | 32.00 | 22.90 |
| Tops of corn, beans, etc., irrigated with copper solutions | | | | 14.00 |
| Beans in soils containing Cu | 9 | 13.0 | 44.00 | 26.00 |
| Squash ditto | 5 | 14.0 | 61.00 | 39.00 |
| Corn ditto | 20 | 4.4 | 239.00 | 42.00 |
| 3. Field samples collected by Lehmann[16] | 43 | 0 | 560.00 | 86.00 |
| Field samples collected by Vedrödi[17] | | | | |
| 1894 | 26 | 40.0 | 1350.00 | 257.00 |
| 1895 | 26 | 10.0 | 680.00 | 151.00 |

patches that may possibly have been due to presence of copper, inasmuch as appearances of this character are sometimes noted as an effect of the application of Bordeaux mixture. Bain states, for instance, that extremely minute amounts of copper stimulate formation of chlorophyll in a cell, and therefore increase the formation of starch.[18] Ewart, also, shows that solutions of copper as dilute as 1 to 30,000,000 prevent the action of diastase upon starch.[19] It is possible, therefore, that the juices of plant tissues containing traces to 239 parts (observed) of copper in 1,000,000 of dry matter may carry sufficient of this amount in solution in the cell sap to hinder the action of enzymes upon starch, and thus prevent its normal translocation.

[16] *Der Kupfergehalt von Pflanzen und Thieren in Kupferreichen Gegenden*, Lehmann Archiv für Hygiene, vol. 27, pp. 1–17, 1896.

[17] Quoted in Brenchley, *Inorganic Plant Poisons*, p. 17, 1914.

[18] Bain, Tenn. Agr. Exp. Sta., vol. 15, Bull. 2, p. 93, 1902.

[19] Ewert, Zeitschr. für Pflanzenkrankh., vol. 14:3, p. 135, 1904.

### Amounts and Significance of Copper in Root Systems

Of far more and unmistakable importance is the effect of copper on root systems of plants. Under all conditions, whether grown in water culture, in pots, plots, or as field crops, the root systems of plants contain much greater amounts of copper than do the aerial portions, as is shown briefly in the following condensation of results:

TABLE XXXI

Summary of Copper Content of Tops and Roots of Plants

| | No. of samples | Cu in p.p.m. Tops | Roots | Ratio |
|---|---|---|---|---|
| Corn, beans, and squash in water cultures, poisoned but living | 3 | 22.00 | 103.00 | 1 to 4.7 |
| Ditto—killed by copper | 3 | 23.00 | 268.00 | 1 to 11.6 |
| Corn grown in soil containing .01 per cent of Cu as $Cu(OH)_2CuCO_3$ | 1 | 6.50 | 152.00 | 1 to 23 |
| Corn grown in soil containing .025 per cent Cu as $Cu(OH)_2.CuCO_3$ | 1 | 21.00 | 728.00 | 1 to 35 |
| Corn grown in soil containing .05 per cent Cu as $Cu_2S$ | 1 | 12.50 | 171.00 | 1 to 14 |
| Bean series grown in soils containing Cu as pptd. carbonate .0025 to 1.5 per cent Cu in soil | 9 | 26.00 | 1431.00 | 1 to 55 |
| Corn series grown in soils containing Cu as $Cu_2S$ .01 to 1 per cent Cu in soil | 7 | 51.00 | 702.00 | 1 to 14 |
| Corn series grown in soils containing Cu as chrysocolla, .05 to 1 per cent Cu in soil | 3 | 13.00 | 266.00 | 1 to 20 |
| Corn series grown in soils containing Cu as pptd. carbonate, .0025 to .05 per cent Cu in soil | 4 | 13.00 | 416.00 | 1 to 32 |

Excluding samples grown in water cultures, the roots of which were cleaned with 4 per cent HCl, probably with loss of some copper, the root systems of experimental cultures contained averages of from fourteen to fifty-five times as much copper as the aerial portions of the plants. Furthermore, fine roots of corn were found in one instance to contain about three times as much copper as coarse roots of the same sample, and, finally, the maximum amount of copper, as determined both by analysis and by observation, in water cultures, was found in the root *tips*

of plants affected by copper. Analyses of water cultures of corn, peas, and wheat showing slight toxic effects gave the following ratios of copper in tops, root systems, and root tips:

| Water cultures showing slight toxic effects | Cu in p.p.m. | | |
|---|---|---|---|
| | Tops | Roots exclusive of tips | Root tips |
| Corn | 27.00 | 91.00 | 545.00 |
| Peas | 17.00 | 1400.00<br>1680.00 | 3428.00 |
| Wheat | 130.00 | 297.00 | 2811.00 |

The root tips in this material, by means of caustic potash (the biuret reaction) and potassium ferrocyanide, show the characteristic purple and dark-red reactions due to copper. In the former case not only copper, but copper *in combination with proteids*, is indicated—the purple color being due to the biuret test, which identifies both copper and proteids simultaneously. In roots grown in water culture, and then subjected to the action of dilute copper solutions, the location of copper in a poisoned root system can be seen under a low power with considerable exactness. The purple of the biuret test begins very definitely with the growing point of the root tip and fades out gradually in comet-like fashion usually within one or two millimeters distance of the tip. New growing points in process of pushing their way through the epidermis along the sides of the roots likewise give a strong but very local biuret reaction. This combination of copper (in the form of oxide) and proteids is one used for the precipitation of albuminoid nitrogen in chemical analysis of feeding stuffs.[20] The amount of copper entering into the combination varies with proteids from various sources. As a rule, animal proteids combine with much less copper than vegetable proteids—averaging about 2.4 per cent of copper for egg albumin. Vegetable proteids combine with from 11.60 to 16.97 per cent of copper oxide and average 11.7 per cent copper.[21] Ordinarily, therefore, a vegetable proteid would be saturated with about one-ninth of its weight of copper; but its physiological activities are disarranged and the root killed by much less than the amount required to saturate the proteid.

20 See Bibliography, p. 488, reference 48.

21 Mann, Chemistry of the proteids, p. 305.

For instance, in samples of wheat and pea roots grown in water culture, it was found by means of nitrogen and copper determinations, using the factor 11.7 per cent copper for saturation of albuminoids, that in wheat roots 4.96 per cent of the copper required for saturation was present and in pea roots 7.99 per cent.

It appears, therefore, first, that copper attacks plant proteids at the most delicate and vulnerable points in the whole plant organization—the growing points of the root systems; and, second, that a small proportion of the copper required for complete reaction is sufficient to kill the protoplasm at these points. Again, it is to be observed that, especially in the seedling stages of growth, the number of growing points is small so that only extremely minute amounts of copper are required to arrest the growth of root tips, the spread of root systems and the nutrition of the plant.

Inasmuch, also, as plants vary greatly in the physical structure and the physiological activity of their root systems, including the number, delicacy and absorptiveness of their growing points, it is not unlikely that the varying sensitiveness to copper salts of different plants, and of the same plant at different ages, may be explained by these observations. Corn, for instance, the most sensitive plant worked with, is characterized in its seedling stages by a small number of vigorously absorptive growing points.

By means of the more delicate dark-red potassium ferrocyanide test, copper may usually be traced through the vessels of the root systems for considerable distances, showing that it is through these channels that small amounts of the metal finally reach the stems and leaves. Here the maximum amounts of copper are found in the outer and upper portions of the plant, where evaporation is most active, and where the greatest residuum of copper therefore occurs. The potassium xanthate (yellow) and hydrogen sulphide (brown) tests also reveal copper in root structures but are not so satisfactory for this purpose as potassium ferrocyanide. (See plate 9.)

The above described reactions, which are so conspicuous in water-culture material killed by copper, are very obscure or imperceptible in roots grown in soils containing copper. The

first material, however, is dead and more nearly saturated with copper; while living roots from soil culture, with proteids combined to but a small per cent of their capacity for copper, do not give satisfactory color tests. These reactions, therefore, do not serve for qualitative determinations of toxic effects in field material.

## Relations Between Amounts of Copper in Root Systems and Injury to Plants

An effort to establish relations between the amounts of copper in parts per million of dry matter in root systems, and toxic effects as shown in the condition of aerial portions of the plant, was only partially successful; but a sufficient number of observations on samples of sufficient size produced under carefully regulated conditions would probably establish such relations. In the tables shown on the preceding pages there is a fair degree of agreement between the members of each experimental series, the copper found in root systems increasing in most cases with the amount of copper in the soils of each particular series of cultures. In the case of beans and corn grown in cultures containing copper in the form of precipitated carbonate, beans show a somewhat higher resistance to toxic effects and also contain larger amounts of copper in the root systems throughout the series. The conditions under which the samples were grown seem to have, within limits, more effect upon the copper content of root systems than the amounts of copper in the soil, as is indicated in the following tabular statement:

TABLE XXXII

Toxic Concentrations of Copper in Soils and Root Systems

| Culture | Points at which toxic effects begin | Cu in root system at point *not* showing toxic effects p.p.m. | Cu in root system at point showing toxic effects p.p.m. |
|---|---|---|---|
| Corn, seven samples from field soils | | 42 at .07% | |
| Corn in field plots containing Cu as sulphate | .04% | 245 at .025% | 296 at .05% |
| Corn in pot cultures containing Cu as carbonate | .023% | 748 at .02% | 509 at .025% |
| Beans in pot cultures containing Cu as carbonate | .035% | 950 at .025% | 1358 at .05% |

In this statement, for instance, field samples of corn roots grown in soil containing 0.07 per cent of copper contained only 42 p.p.m. of copper in dry matter, while a plot sample grown in soil containing .025 per cent of copper contained 245 p.p.m. of copper in dry matter, and corn grown in pot culture containing 0.02 per cent of copper in soil contained 748 p.p.m. of copper in dry matter.

These differences may be due to the coarser root systems of plot and field-grown samples, this condition being associated with relatively small amounts of copper in dry matter. In view of the great labor involved in preparing root samples for analysis and the very variable results obtained from copper determinations made upon such material, there seems to be little hope of establishing satisfactory ratios of copper to dry matter for the purpose of determining that a sample of field material has been injured by copper. It is probable, however, that for comparative purposes, pot cultures of field soils conducted under uniform and carefully regulated conditions, with standard plants of known behavior, may yield figures of comparative value in determining the character, toxic or otherwise, of a soil containing copper. Corn is an excellent summer-growing plant for the purpose, inasmuch as it shows toxic effects easily, grows rapidly, and affords abundant root materials for analytical determinations. For winter cultures, wheat serves well. Both plants are representative of standard crops for the region under discussion.

## Pathological Effects

Pathological effects in tops and roots may confirm to a considerable extent, the fact that a plant has been poisoned by copper. The lengthwise yellow striping of corn and wheat leaves due to toxic amounts of copper is not distinctive since the same appearances may result from various other conditions inducing malnutrition, such as those mentioned on a preceding page. Usually, however, careful observation will identify or eliminate these other disturbing factors.

Root systems grown in coppered soils are also conspicuously injured, being stunted in growth, of harsh and crinkly texture

and (in the case of corn) showing characteristic proliferated root tips. The epidermis is thick and rough and the cells in longitudinal tangential section contract from the oblong toward the circular form. Here, again, other factors, such as alkali salts in excess, may lead to similar appearances; and these must be eliminated in a diagnosis of copper injury.

## Soil Conditions Relating to Toxic Effects of Copper upon Plants

Certain conditions favor, others oppose the toxic action of copper under field conditions, the general tendency being to modify or do away with toxic effects, where the amounts of copper are not excessive.

*Carbon dioxide* in the soil, alone and in conjunction with certain salts ($NaCl$, $Na_2SO_4$) tends to form solutions of basic copper carbonate. Carbonates ($Na_2CO_3$,$CaCO_3$) lessen the solubility of basic copper carbonate in carbon dioxide and, therefore, the toxicity of copper compounds in soils containing these carbonates.[22]

*Coarse, sandy soils* favor toxicity by permitting free movement of solutions and because the withdrawal in them of copper from solution by physical and chemical reactions is minimum.[23]

*The character of the compound of copper* to which roots are exposed is important. In pot cultures of precipitated carbonate of copper, of sulphide in the form of chalcocite pulverized to go through a 100-mesh sieve, and of silicate in the form of chrysocolla pulverized to 100-mesh, toxic effects appeared with corn as follows:

Pot culture of corn; Cu in form of pptd. carbonate—showing toxic effects at .023% Cu in soil

Pot culture of corn; Cu as chalcocite, 100-mesh—showing toxic effects at .08% Cu in soil

Pot culture of corn; Cu as chrysocolla, 100-mesh—showing toxic effects at .08% Cu in soil

The precipitated carbonate is not only more soluble in carbon dioxide than in chalcocite, but also more easily acted upon

[22] See Bibliography, p. 487, reference 12.

[23] See Bibliography, reference 18.

by the acids of plant roots than chalcocite or, probably, chrysocolla. Under field conditions, copper in tailings is originally mostly in the form of sulphides, chiefly chalcocite, which oxidizes only slowly to sulphate in presence of water and air. Chalcocite, 3.2 grams, shaken up with 600 c.c. of water, and air, for twenty-eight days, yielded only 16 mg. of soluble copper. The soluble sulphate in contact with silicates and carbonates of the soil is converted to insoluble forms. The process is gradual and the amount of soluble copper present at any one time is small.

*The tilth of the soil* is significant. A pot culture very thoroughly mixed with 0.1 per cent of copper as carbonate resulted in badly poisoned plants containing about four times as much copper in root systems as in a lumpy mixture of soil containing the same amount of copper. The heavy tailings clay, with which copper is chiefly associated in the district studied, tends to remain in lumps and masses, thus minimizing toxic effects of contained copper compounds.

*In water cultures* toxic effects of copper salts are lessened by salts contained in well-water or in nutrient solutions. This is due, in part, to the presence of other ions, the effect of which is to decrease the ionization of copper salts, with consequent decrease in toxicity. This observation applies to soil-water solutions which contain considerable amounts of alkali salts. It is of interest in this connection to note that certain combinations of salts representing complete mineral nutrients exert maximum antitoxic action to copper salts;[24] and that therefore a fertile soil containing maximum amounts of plant nutrients will tend to minimize toxic effects of copper.

*Antagonistic solutions,* so called, involving copper, may also account for a decrease in toxicity. By reason of a property of the semipermeable membranes of root systems, ions may be either more readily or less readily allowed to penetrate. When penetration is decreased through the addition of ions of other soluble salts this salt is said to be antagonistic in character. Copper is thus antagonized by sodium and potassium salts, of which the soluble salt content of the soil is chiefly composed.[25]

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[24] A. Le Renard, Essai sur la valeur antitoxique de l'aliment complet et incomplet. Abstracted in Science n. s. vol. 28, no. 712, p. 236, 1908.

[25] See Bibliography, p. 488, references 35–44, 5?

*Physical attractions,* or adsorptive effects, also account for a very considerable lessening of the amount of dissolved copper salts, in contact with soil particles. Jensen, for instance, finds that a dilute copper solution is ten times as toxic in the free condition as when it is mixed with an artificial quartz soil, that is to say, the quartz reduces the toxic effects about nine-tenths. Inasmuch as the reduction in toxicity is a function of the solid surface to which the soluble salts are exposed, the finer the state of division of a soil the more will be the adsorption and the less will be the toxic effects of a stated copper solution.[26]

*The age of plant roots* markedly affects their susceptibility to copper salts. Young and tender roots, containing large amounts of protoplasm, are much more quickly and easily poisoned than old and comparatively fibrous structures containing a small proportion of protoplasmic materials. This may be due to differences in the thickness of cell walls protecting the cell contents from outside substances; it may be due to a different degree of permeability of the protoplasm of older roots to copper salts; or it may be due to lessened reactivity due to changed chemical character. In any case, this observation indicates a distinctly greater resistance to copper in soils, of older, more fibrous, and possibly intrinsically more resistant root systems. Different species of plants also show varying degrees of resistance to copper salts. In pot cultures, peas are distinctly more resistant to precipitated carbonate of copper than corn. Different plants of the same species also show a certain amount of individuality with reference to absorption of copper.

## Stimulation

Not only do the various influences described above lessen the toxic effects of copper upon plants, but it is possible, also, that the amounts of copper may be decreased in the field to the point at which stimulating effects occur. As shown in the discussion of water cultures on preceding pages, extreme dilutions of copper salts in distilled water, for instance, 1 part to 100,-

[26] G. H. Jensen, Botanical Gazette, vol. 43, p. 11, Jan., 1907.

000,000, caused increased growth of root tips growing in these solutions. This observation accords with those of some other experimenters, not only with copper solutions but with solutions of various other metals, and bears a certain analogy to stimulating effects upon animals observed with very small amounts of poisons, such as arsenic and strychnine. Stimulation was also observed in the case of certain pot cultures watered with dilute copper solution in such a way that these solutions were filtered through a thin layer of soil before they reached the plant roots. Under these conditions a portion of the root systems must come in contact with extremely dilute copper solutions residual from the reactions of copper salts with the soil. As in the case of water cultures, these extremely dilute solutions must have exerted the stimulating effects which were apparent in several cultures made in this manner.

In the case of pot cultures also, in which stated amounts of copper were uniformly mixed throughout the soil, apparent stimulation of growth was occasionally observed; for instance, with 0.01 per cent of copper in the form of precipitated carbonate in a culture of corn.

A satisfactory explanation of stimulation effects is not available. It is to be supposed that stimulation in a soil culture in which copper sulphate is used may be explained by the action of the $SO_4$ ion upon the soil in releasing plant food for the use of the plant. However, such stimulation is seen in water cultures where this does not occur. Lipman[27] has observed that under certain conditions the nitrifying flora of soils is stimulated by salts of copper, zinc, iron and lead. Such stimulation, through increased elaboration of nitrates, may account for the behavior of cultures showing increased growth. Stimulation effects, therefore, which undoubtedly occur both in water and in soil cultures, are perhaps due to more than one different cause—to chemical and bacterial agencies in soils, and to a pathological disturbance in water cultures.[28]

Taking into account the very minute amounts of copper salts with which stimulated growth is associated, and the very gradual

[27] Lipman, C. B., and Burgess, P. S., Univ. Calif. Publ. Agr. Sci., vol. 1, no. 6, pp. 127–139, 1914.

[28] See Bibliography, pp. 487–488, references 2, 4, 27, 33, 53, 45.

addition of copper to new ground that may occur through irrigating waters, it is not impossible that in favorable situations an actual increase in vegetable growth in the field due to copper may take place; but it is not possible in the field to prove this supposition because of many other factors, the effects of which prevent trustworthy observation.

## Field Observations

In view of the many factors influencing results in the field, some leading towards toxic copper effects, some opposing toxic effects, and still others pointing to the possibility of stimulated growth, it is of interest, finally, to refer to field conditions as they have existed in irrigated lands under the Clifton-Morenci mines for the twelve years during which the district has been under observation. At the beginning of this period, in 1904, considerable accumulations of copper-bearing tailings were evident, more particularly at the heads of alfalfa fields, where they sometimes attained a thickness of as much as ten inches or more. These blankets usually thinned out and disappeared between 100 and 200 feet from the head ditches, leaving crops in lower portions unaffected. Deposits of river sediments were observed in other irrigated districts not affected by mining detritus. The growth of alfalfa was more depreciated by the denser and thicker tailings blankets; and yellow foliage of young grain and young corn was considerably in evidence in tailings, but not as an effect of ordinary sediments. In 1908, the tailings were impounded, and some of the best farmers began the practice of cultivating alfalfa to break up the old accumulations, incorporate them with the soil, and secure better penetration of water and air to the roots of the crop. Following this procedure the stunted growth at the heads of alfalfa lands has considerably but not yet entirely recovered. Patches of yellow young barley, wheat, and oats are still to be observed on old tailings deposits; but as the plants become older they become normal in appearance, and yield apparently normal crops. These observations, which may be repeated many times in the course of a day's reconnaissance in the district, from May to September for alfalfa, and February

to May for grain, may be explained by the following considerations: The wedge-shaped deposit of tailings indicated in the diagram (fig. 15) at first so obstructed access of water and air to alfalfa root-systems that only stunted development was possible either of roots or tops. With an annual cultivation of this blanket and the incorporation of river sediments and better penetration of irrigating waters, deleterious effects tend to disappear and the crop again approaches normal.

Similar land when plowed for grain contains most of the copper associated with old tailings at the surface of the soil. Young grain, therefore, with shallow and susceptible root sys-

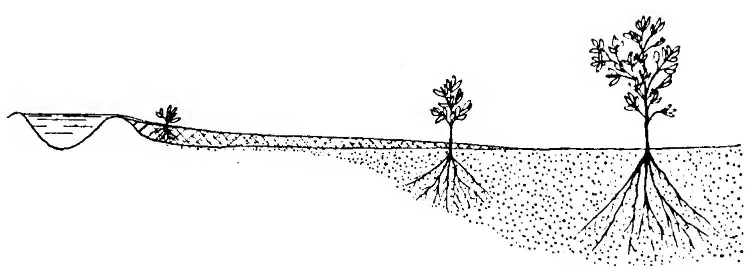

Fig. 15.—Diagram showing behavior of root systems under influence of tailings blanket.

tems, at first, if ever, shows effects of copper in the soil, recovering as root systems penetrate to greater depths where they encounter uncontaminated soil.

## Effects of River Sediments

With reference to the further trend of copper effects upon vegetation in the district, assuming the permanent exclusion of solid tailings but a constant addition of about one part of copper to one million of irrigating water used, it is of interest to take into account the diluting effect of river sediments upon copper compounds in the district.

In four acre-feet of Gila River water, these sediments will amount to about eighty tons per acre a year,[29] of which amount the ten pounds of copper contributed in irrigating waters is only 0.006 per cent.

[29] Forbes, R. H., Ariz. Agr. Exp. Sta. Bull. 53, p. 61.

Irrigating sediments alone, therefore, considered in their general relation to amounts of copper which cannot be prevented from reaching irrigated fields, are sufficient in quantity to reduce ultimately the amounts of copper observed below 0.01 per cent in the soils of this district. Since 0.01 per cent is a safe minimum, river sediments, alone, incorporated with the soil are probably sufficient to ameliorate gradually existing accumulations of copper salts and to take care of further contributions in soluble form which cannot at present be avoided.

## Effect of Cultivation upon Alfalfa

Finally, it is of interest to observe the improvement in a field of alfalfa, in the district studied, between the years 1905 and 1916.

June 23, 1905, the writer carefully measured, cut and weighed a representative plot of alfalfa in William Gillespie's field near Solomonville, Arizona. This field was suffering from an accumulation of tailings, the depreciation in yield at the upper ends of alfalfa lands being conspicuously evident. Following the exclusion of tailings from the irrigating supply in 1908, and with a cultivation each winter with a disk or a spring-tooth harrow, the condition of the field gradually improved until, June 13, 1916, the writer returned and again measured, cut, and weighed the identical plot of alfalfa that had shown bad effects eleven years before. Following are the data, with diagrams, relating to these two cuttings of alfalfa, which are representative for the district within which tailings were deposited.

1. *Alfalfa seriously affected by tailings, June 23, 1905.*

Three lands in William Gillespie's field east of house, near Solomonville, under Montezuma Ditch, out of Gila River. A good stand of alfalfa five years old. Heavy adobe soil; field never disked.

The three lands observed were, over all, 95 feet wide, and divided into plots 100 feet long from top to bottom of field. Ten feet next the ditch was discarded because of banks and bare spots, and the extreme lower portion of the field because of roadways. A portion of plots 6 and 7 was discarded on account of Johnson grass.

Observations were made June 23, 1905, on the second cutting, just beginning to bloom, the field having been irrigated twice since the last cutting. After stirring and raking, the yield of dry hay was weighed June 24. Weather very hot and dry. Following are the data relating to this series:

| Plot | Dimensions in feet | Height of alfalfa, inches | Yield of plot, pounds | Tons per acre | Depth of tailings on plot, inches | Condition of surface soil at time of cutting |
|---|---|---|---|---|---|---|
| 1 | 95x100 | 19 | 240 | .69* | 1½–3½ | Dust-dry and somewhat cracked |
| 2 | 95x100 | 20 | 340 | .87* | 1 –2 | Dry and badly cracked |
| 3 | 95x100 | 23–25 | 570 | 1.31 | ¾–1¼ | Dry, cracked at upper end |
| 4 | 95x100 | 24 | 595 | 1.36 | ½–1 | Moist, not cracked |
| 5 | 95x100 | 23 | 550 | 1.26 | ¾–1 | Moist, not cracked |
| 6 | 60x100 | 28 | 400 | 1.41* | ½–1 | Moist, not cracked |
| 7 | 60x100 | 27 | 430 | 1.48* | ⅓– ½ | Moist, not cracked |

* Corrected for thin stand and trash.

2. *Alfalfa slightly affected by tailings, June 13, 1916.*

The same three lands, continuously in alfalfa since 1905. A perfect stand, thin spots reseeded by means of a seed crop in 1915. The field had been spring-tooth harrowed each winter for about ten years, especially at heads of lands, to break up the tailings blanket and secure better penetration of irrigating water.

As in 1905, ten feet next the ditch was discarded, also the extreme lower portion of the field. Johnson grass had nearly entirely disappeared.

Observations were made June 13, 1916, on the second cutting, just beginning to bloom, the field having been irrigated twice since the last cutting. After raking and piling, the dry hay was hauled and weighed June 17. The weather was moderately hot and dry; and conditions generally the same as those under which the crop was cut in 1905. Following are the data for the second series of observations:

| Plot | Dimensions in feet | Height of alfalfa, inches | Yield of plot, pounds | Tons per acre | Appearance of tailings | Condition of soil at time of cutting |
|---|---|---|---|---|---|---|
| 1 | 95x100 | 36–21 | 875 | 2.00 | Distinct | Surface dusty, drier soil |
| 2 | 95x100 | 22–34 | 857 | 1.96 | Distinct | Surface dusty, drier soil |
| 3 | 95x100 | 34–36 | 972 | 2.22 | Slight | Moist throughout |
| 4 | 95x100 | 30–36 | 910 | 2.08 | None | Moist throughout |
| 5 | 95x100 | 31–33 | 900 | 2.05 | None | Moist throughout |
| 6 | 95x100 | 28–36 | 860 | 1.96 | None | Moist throughout |
| 7 | 95x100 | 34–37 | 870 | 1.98 | None | Moist throughout |
| 8 | 95x100 | 33–36 | 910 | 2.08 | None | Moist throughout |

Comparing these two statements, and illustrating them by means of the following diagram (fig. 16), it is evident that the depreciation in yield observed in the upper plots in 1905 has disappeared in 1916, the yields on the last date being practically uniform from top to bottom of the field. Effects of tailings are still plainly visible in plots 1 and 2 in spots and patches of short alfalfa, compensated for, however, by areas of stimulated growth apparently due to seepage from the adjacent ditch. The yield of the field as a whole is also much improved due to cultivation and reseeding of the field.

In brief it may now be stated that, following the exclusion of tailings from the irrigating waters of this locality, it has been found possible, in this carefully observed case, to overcome the deleterious effects of tailings deposits upon alfalfa, slowly but almost entirely, in about ten years.

Thus, co-operation between miners, in restraining tailings from irrigating streams, and those farmers who cultivate their alfalfa intelligently, effectually disposes of the most serious problem that has arisen in connection with copper-mining detritus.

The chemical composition of tailings, in fact, would indicate that, as in the case of humid region subsoils, when they are enriched by the addition of organic matter and nitrogen, and filled with bacterial life, they may make very good soil. Following is a statement of the composition of four representative samples of ores and tailings, with reference to potash and phosphoric acid:

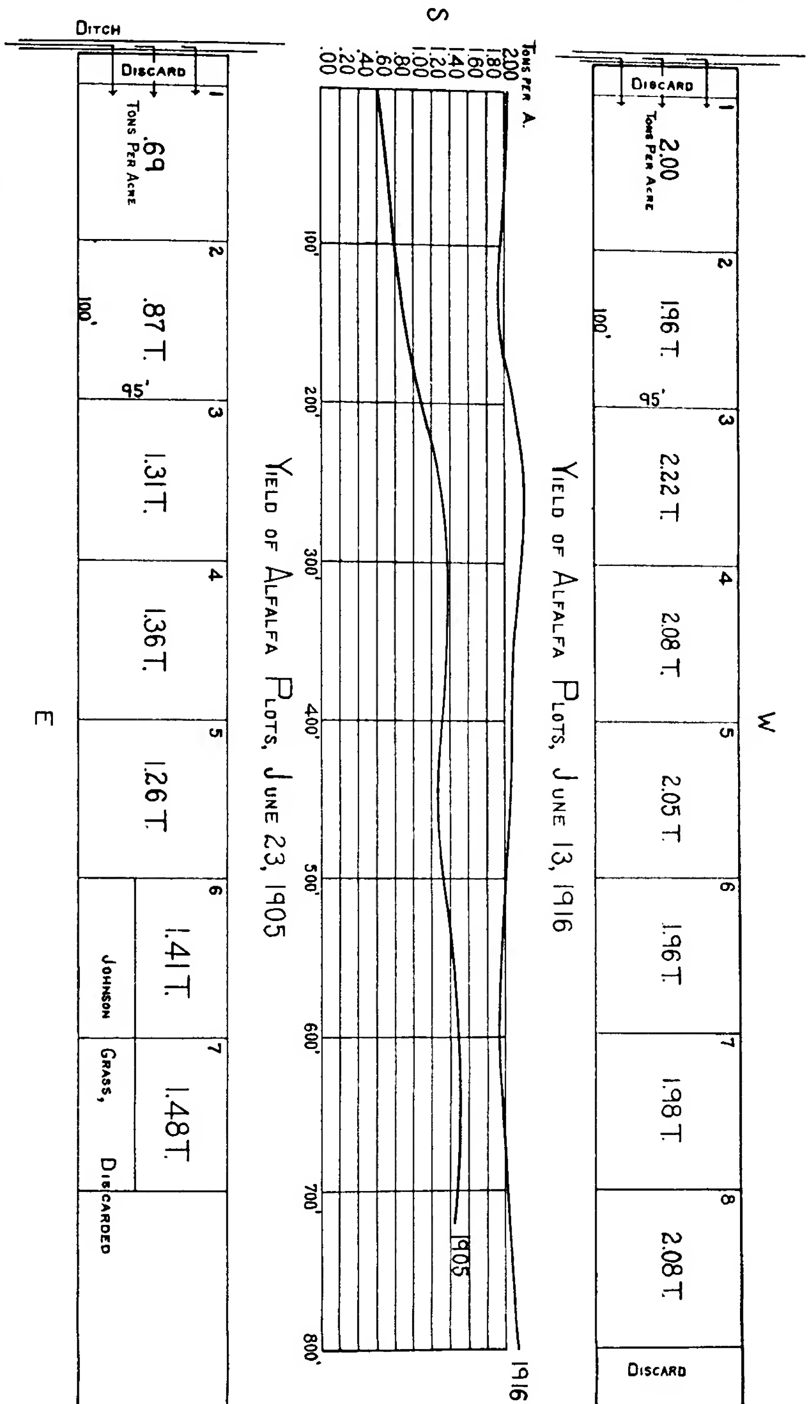


Fig. 16.—Diagram showing yields of alfalfa from head to foot of a land damaged by tailings in the field of Wm. Gillispie, east of house, June 23, 1905; and the same land June 13, 1916, tailings having been kept out of the water since 1908, with the field disk-harrowed annually.

| Sample No. | | Potash $K_2O$ | Phosphoric acid $P_2O_5$ | Nitrogen N |
|---|---|---|---|---|
| 3491 | Sulphide ore | .64% | .11% | Doubtful |
| 3492 | Oxidized ore | .44 | .11 | |
| 3438 | Sulphide tailings | .79 | .29 | traces |
| 3439 | Oxidized tailings | .67 | .12 | |

These ores and the tailings derived from them are rich in potash, and contain unexpectedly large amounts of phosphoric acid; but nitrogen is almost nil.

## SUMMARY

1. Copper is shown, as a direct effect of the Clifton-Morenci mining operations in Arizona, to be distributed throughout water-supplies, soils, the vegetable and the animal life of an underlying irrigated district.

2. Smaller amounts of copper are found elsewhere in the State where the drainage basin includes mining operations or ore-bearing areas.

3. Individual plants grown in water cultures or in soil containing copper show a comparatively small, and probably not injurious, accumulation of copper in the aerial portions of the plants; but the root systems, carefully cleansed of externally adhering copper, contain relatively great amounts.

4. Copper in root systems, as shown by the biuret test, is largely in combination with plant proteids, especially at the growing points of root systems and near vicinity. The place and nature of the reaction accounts for the extreme toxicity of copper salts to plants. The varying sensitiveness of plants to copper salts may possibly be explained in part by the number and disposition of exposed growing points.

5. Conditions favoring toxicity of copper compounds are the presence of carbon dioxide and certain soluble salts which assist in forming copper solutions that come into contact with plant roots; coarse, sandy soils favoring free access of copper solutions to plant roots and minimizing the withdrawal of copper from solution by adsorption; and the presence of copper in the form of the more soluble precipitated carbonate.

6. Conditions opposing toxicity of copper compounds are the presence of copper in the form of chrysocolla and chalcocite; adsorption through contact with finely divided soil particles; reactions with carbonates, silicates, and organic matter tending to precipitate copper from its solutions; the presence of certain soluble salts in the soil that overcome toxic action; and increased resistance of old plant roots.

7. The stimulation by copper of vegetative growth in pot and water cultures has been observed. Stimulated growth of crops under field conditions is a possibility.

8. Pot cultures may be used for comparative determinations of toxic effects upon plants of copper in soils, if conducted under rigidly uniform conditions. The copper content and the physiological response to copper of such material will be much greater than for similar cultures grown under plot or field conditions.

9. Copper injury in field soils containing doubtfully toxic amounts of copper may be diagnosed by a combination of symptoms. Facts which indicate such injury in a soil containing 0.1 per cent of copper (more or less) are: yellow tops (for winter grains) in absence of other conditions that cause yellow tops; crinkly root systems (in absence of excessive amounts of alkali salts); and a high copper content in dry matter of root systems. Combined evidence of this character, which may be observed in the district studied, indicates toxic copper effects.

10. Field observations before and following the exclusion of tailings from the irrigating water-supply indicate that conditions in the district studied are gradually improving, due to the cultivation of alfalfa and to the incorporation of river sediments with accumulations of tailings. Noticeable toxic effects in the field exist only where the roots of young, growing crops are exposed to surface soils containing maximum amounts of copper. The general tendency in the district is probably toward decreasing rather than increasing percentages of copper in irrigated soils.

11. Methods of analysis have been developed for the purpose of determining reliably small amounts of copper in vegetative material, particularly in root systems of plants grown in soils containing copper.

# Part III.—APPENDIX

## METHODS OF ANALYSIS

WITH THE COLLABORATION OF E. E. FREE AND DR. W. H. ROSS

Freedom of samples, especially vegetation, from contamination with adhering copper; and accurate methods for determining minute amounts of copper in sediments, soils, waters and vegetation, are vital to the integrity of the work recorded in this publication.

Unusual care was taken to perfect methods for preparation of samples, especially roots grown in media containing copper; and refined manipulation in the determination of copper reduced the limit of error to approximately .00001 gram, or .01 milligram.

### REAGENTS AND APPARATUS

*Distilled water* of three derivations was used: (1) University of Arizona well water very slowly distilled through a block-tin worm; (2) the same, redistilled from glass; and (3) University of Arizona well water distilled from glass.

*Nitric and sulphuric acids* from Baker & Adamson were used.

*Ammonia and $H_2S$* employed were passed through two wash bottles.

Blank determinations from time to time with reagents employed gave no trace of copper, thus insuring results obtained by means of them.

Copper was determined by electrolysis, in minute amounts according to the manipulation of E. E. Free.[1]

The balance used was a No. 2112 Eimer and Amend short-beam assay balance, "distinctly sensitive to 1/200 milligram."

### MANIPULATION

*Ores and tailings.*—1–2 gms. were digested with a mixture of 8 c.c. $HNO_3$ and 5 c.c. HCl on a hot plate, then 4 c.c. $H_2SO_4$ added and evaporated to $H_2SO_4$ fumes (method used in Old Dominion laboratory at Globe, and Copper Queen at Bisbee). Took up with water, filtered, neutralized with ammonia, then added 2 c.c. $H_2SO_4$ and a few drops of $HNO_3$ and electrolyzed.

*Soils.*—Soils were examined by two methods:

(*a*) 100 gms. soil was treated with a mixture of 80 c.c. $HNO_3$ and 20 c.c. $H_2SO_4$ and digested in a porcelain dish on a hot plate to sulphuric fumes; digested with 200 c.c. water, filtered, washed up to about 500 c.c., evaporated to 200 c.c. precipitated iron with ammonia, filtered, washed with about 500 c.c. water, alkaline filtrate reduced by evaporation, acidified faintly with HCl and $H_2S$ passed for half an hour. The faint black precipitate was

[1] Electrolytic determination of minute quantities of copper, 12th Gen. meeting Am. Electrochem. Soc., October 17–19, 1907.

allowed to settle several hours, then filtered, and the precipitate, including filter, digested with 5–10 c.c. $HNO_3$ and water until copper was dissolved, solution filtered, a few drops of $H_2SO_4$ added, evaporated to fumes, and copper determined by electrolysis with addition of 5–25 drops of $HNO_3$.

(*b*) 200 gms. soil was digested as above with $HNO_3$ and $H_2SO_4$, evaporated to fumes of $H_2SO_4$, digested with water, filtered and washed up to 500 c.c., made alkaline with ammonia and made up to 1000 c.c. After settling, 500 c.c. or 100 gms. aliquot, was filtered off and copper determined as in (*a*).

*Waters.*—Waters were evaporated to dryness, the residue digested with sulphuric acid and water, filtered hot, excess of $H_2SO_4$ evaporated, filtered into platinum dish, a few drops of $HNO_3$ added, and electrolyzed.

*Vegetation.*—Air-dried samples were burned in a small sheet-iron stove, the iron of which was found to contain *no trace* of copper. Two samples of mistletoe, difficult to burn, were reduced in a *new* muffle in gasoline assay furnace. The charred and partly burned material was moistened with water, and concentrated $HNO_3$ added (100 to 200 c.c.) until effervescence ceased, digested until in plastic condition, diluted with hot water and filtered. Evaporated bulky filtrate to dryness, took up with water and $HNO_3$, filtered (getting rid of much organic matter), added about 20 c.c. $H_2SO_4$, evaporated to $H_2SO_4$ fumes, driving off all but about 5 c.c. $H_2SO_4$, added water, filtered off insolubles, made up filtrate to about 500 c.c., passed $H_2S$, and proceeded as usual for copper.

The completeness of the extraction of copper from vegetation by the above method was verified as follows: The extracted, charred residue from 2 lb. 8 oz. of dry corn leaves and blooms in which 1.32 parts Cu per million was found (Sample 3529) was removed from filter paper after washing, moistened with $H_2SO_4$ and additionally burned in a porcelain dish, being finally reduced, after again moistening with $H_2SO_4$, in a platinum dish in the muffle. The resulting pink ash was then fused with three parts of dry $Na_2CO_3$ (Kahlbaum) and poured on clean porcelain. The fusion was soaked in water with addition of $H_2SO_4$, evaporated nearly to dryness, filtered from insoluble portion (lime, salts, etc.), again evaporated and filtered, and a third time the same, finally driving off excess of $H_2SO_4$ and electrolyzing as usual. A black precipitate of carbon but *no Cu* was obtained, the same being true of a blank determination on the $Na_2CO_3$ used.

Roots of plants grown in water cultures or in soils must be most thoroughly cleansed of externally adhering copper, since this will introduce excessive errors where the content of copper is small. Three methods of preparing roots for copper determination were employed:

1. Roots grown in water cultures containing copper were dipped for about ten seconds in 4 per cent HCl, immediately

washed in copper-free water and dried. Careful observation indicated that adhering copper salts deposited from water solution were completely removed by this treatment. It is probable that the acid penetrates plant tissues somewhat in the time employed and removes some copper. The results are, therefore, probably severely conservative.

2. Roots grown in soil cultures containing copper cannot be safely cleansed with HCl, which does not readily dissolve silicates and sulphides of copper, and which cannot be allowed to remain in contact with plant roots for more than a few seconds.

Carbon dioxide in water was finally selected as a mild, slow but finally effective solvent for the purpose. Samples of roots were first very thoroughly washed in copper-free well-water, then placed in five-liter jars with ground glass covers, a stream of washed $CO_2$ passed, the jars shaken and treatment with $CO_2$ repeated until the water was saturated, then allowed to stand with occasional shaking for twenty-four hours. The solution was then siphoned or filtered off and the treatment repeated until, on evaporating the bulky filtrates, no more copper was found. To prevent putrefaction during long-continued washings, a pinch of thymol was added to each washing. From nine to thirty-one washings were found necessary to cleanse plant roots thoroughly, the process being laborious and time-consuming. When the sample yielded no more copper to wash waters it was dried, burned and copper determined according to the method for small amounts in plant ashes.

Following is a record of washings for examples of roots cleaned by this process:

(1) Corn roots grown in a pot culture of soil containing 0.01 per cent of copper as basic carbonate.

| | $H_2S$ test | | Quantitative by electrolysis |
|---|---|---|---|
| First wash | distinct | | |
| Fifth wash | distinct | | |
| Ninth wash | doubtful | 1 liter of filtrate | no Cu |

(2) Corn roots grown in a pot culture of soil containing 0.05 per cent copper as $Cu_2S$.

| | | Quantitative by electrolysis |
|---|---|---|
| Tenth wash | 2 litres of filtrate | .00006 gm. Cu |

(3) Barley roots from field soil containing tailings.

| | | Quantitative by electrolysis |
|---|---|---|
| First wash | 2.433 litres of filtrate | .00035 gm. Cu |
| Second wash | 2.531 | .00012 |
| Fifth wash | 2.22 | .00009 |
| Sixth wash | 2.41 | .00004 |
| Seventh wash | 2.00 | .00002 |
| Eleventh wash | 2.00 | .00000 |

(4) Coarse roots of field corn grown in soil containing tailings.

| | $H_2S$ test | Quantitative by electrolysis |
|---|---|---|
| Twenty-fifth wash | distinct | |
| Twenty-ninth wash | | .00005 gm. Cu |
| Thirty-first wash | | .00000 |

Samples vary as to number of washings required to remove the last trace of copper, but the definiteness with which, finally, copper usually ceases to be extracted by $CO_2$ water indicates completeness of the operation. This is further emphasized by the comparatively large amounts of copper which are then found in root systems thus cleansed.

3. A third method of preparing roots for copper determination, involving less labor than by washing in $CO_2$ water, is as follows: Cleanse roots thoroughly in clean water with a camel-hair brush, dry, burn and weigh the ash, then estimate total copper. Determine copper in soil shaken from sample, assume ash as all soil and deduct copper in this amount of soil from total copper found in ash. Results by this method are low, but not seriously in error if sample is thoroughly washed.

| Example | Dry matter | Ash | Gms. Cu | Pts. Cu per million |
|---|---|---|---|---|
| Sample 2*a* grown in soil containing 0.05% copper | .3561 gm. | 10.84% | .000115 | 322 |
| Ash in sample | .0386 | | | |
| Copper in ash assumed as soil | | | .000019 | |
| Net copper assumed | | | .000096 | 270 |

The correction introduced reduces parts per million of copper from 322 to 270, which latter figure is conservative in character.

Of the three methods above described, No. 2 is undoubtedly most exact, but is extremely laborious and time-consuming.

## THE DETERMINATION OF COPPER IN SMALL AMOUNTS OF PLANT ASHES

The ash is placed in a platinum dish without previous pulverization and moistened with concentrated sulphuric acid in sufficient quantity to bring all parts of the ash in intimate contact with the acid. The material is then thoroughly stirred and heated on a sand bath until fumes of $SO_3$ begin to come off, then allowed to cool and a sufficient quantity of hydrofluoric acid added to bring the acid in contact with the whole mass, then allowed to stand for at least half an hour and again heated until

$SO_3$ fumes come off. The material is now washed into a casserole, moistened with sulphuric and nitric acids and digested at a low heat for at least one hour. The heat is then increased until $SO_3$ fumes are again driven off. The mass is moistened with three to four times its bulk of distilled water and digested at a gentle heat from one to two hours, filtered hot and then the filtrate and washings evaporated almost to dryness, thus driving off the excess of sulphuric acid. The resulting residue is taken up with hot water and again filtered to separate the solution from precipitated calcium sulphate. This evaporation and filtration may have to be repeated one, two or three times in order to get the solution sufficiently free from calcium sulphate. The final filtrate, which contains the copper, is then diluted to about 150 to 200 c.c. in a tall beaker, a small quantity of hydrochloric acid is added and hydrogen sulphide passed until the solution is thoroughly saturated. During the hydrogen sulphide precipitation there should be no nitric acid or nitrates present in the solution. A large quantity of organic matter is also disadvantageous and may be avoided by evaporating the solution several times to dryness with nitric and sulphuric acids, finishing finally with an evaporation with sulphuric acid alone in order to drive off all traces of nitric acid.

The precipitate from the treatment with hydrogen sulphide is filtered off, washed with water saturated with hydrogen sulphide and digested with a small quantity (2 to 5 c.c.) of nitric acid in a casserole. The digestion should be begun cold and the heat gradually increased. If the digestion is begun at a high temperature the sulphur formed by the decomposition of the copper sulphide will form a film of molten sulphur around the granules of copper sulphide, and this tends to prevent their solution in nitric acid. The precipitate after digestion in nitric acid should be a clear green or else a yellow. If there is any trace of dark color, brown or black, it means that either organic matter has been precipitated with the copper sulphide precipitate, which is extremely unlikely, or else that the above-mentioned sulphur film has formed around some of the particles of copper sulphide preventing their solution in the nitric acid. If the latter be the case, the determination may still be saved by placing the precipitate in a platinum dish and heating over a gentle flame until the sulphur is volatilized. The residue of copper sulphide or of copper oxide may then be digested in nitric acid. The digestions in nitric acid should not be carried to a heat high enough to decompose the copper nitrate formed by the solution of copper sulphide.

After digestion in nitric acid and the evaporation of any large excess of nitric acid, the residue is taken up in hot water, acidified to contain 2–4 per cent nitric acid and filtered into a large platinum dish, ¼ to ½ c.c. of sulphuric acid is added, and the solution electrolyzed with a voltage of from 2 to 2½

volts and a current not greater than one ampere. The voltage may be higher than 2½ volts if necessary but should not be high enough to raise the current beyond the limit given. The electrolysis should be continued at least three hours and preferably nine to twelve hours. The dish is, of course, the cathode. When the electrolysis is complete the electrolyte is washed out of the dish by means of the sucking-bottle and the dish is thoroughly washed with distilled water. In case the deposit of copper on the dish is spongy and loosely adherent it is not safe to wash out the electrolyte. In this case the copper should be redissolved and the electrolysis repeated, using a little more sulphuric acid. If the copper still refuses to come down in adherent form the addition of 2 to 5 c.c. of a one per cent solution of gelatine will often assist the precipitation. In case of a stubborn refusal of the copper to give an adherent deposit it is necessary to dissolve it, evaporate to dryness with sulphuric acid, and reprecipitate with hydrogen sulphide, continuing the process from this point as before.

If the copper refuses to come down at all the trouble is probably an excess of acid in the solution. This may be corrected by the addition of a few drops of ammonia. The concentration of acid in the solution must lie between one and five per cent. At least a small part of this should be sulphuric acid as nitric acid will be destroyed in the course of the electrolysis if it alone is present, and the solution may become alkaline (from $NH_4OH$), which will prevent proper precipitation. Chlorides and organic salts, such as acetates and tartrates, should be carefully avoided.

The resulting deposit of copper will probably contain traces of carbon and possibly of platinum. In order to eliminate these and at the same time precipitate copper upon an electrode more suitable for accurate weighing, a second electrolysis is made, using this time the dish as anode and using as cathode a small spiral of platinum wire suspended from a hook of silver (or platinum) wire which in turn is connected to the battery. The electrolysis should also be conducted in nitric and sulphuric acid solution and what is said above as to obtaining satisfactory deposits applies with equal force here. In this case, however, owing to the small surface area of the cathode, it is necessary to work with very much smaller currents than were used in the first electrolysis. The maximum current to be used must be so adjusted by trial as to give bright and adherent deposits. 1–100th ampere and 1.8 volts is a good current for the purpose. It is well to use as the source of current for this electrolysis four Edison-Lalande cells and to have in the circuit a resistance of from 30 to 80 ohms. This gives an electromotive force at the dish of about 1.8 volts. Two determinations may be run in parallel. In this case it is not permissible to use a gelatine solution in order to secure satisfactory deposits, as the copper will be slightly contaminated with gelatine and the obtained weight will be too high.

The electrolysis should be run at least nine hours. When completed, the electrolyte should be washed out as before without breaking the current, the electrode lifted from the solution, disengaged from the supporting hook, and washed and dried by dipping successively in water, alcohol and ether and placing in a desiccator over sulphuric acid. After having remained in the desiccator for an hour the electrode is ready for weighing. Weighings should be made on an assay (button) balance adjusted to maximum sensibility. After weighing, the copper is removed from the electrode by dipping in concentrated nitric acid, and the electrode cleaned and dried by dipping successively in distilled water, alcohol and ether and placing in a desiccator. It is again weighed as before and the difference of the two weights gives the copper obtained.

The electrolyte (from each electrolysis) which has been washed out of the dish by means of the suction flask, is evaporated to dryness taken up with water, acidified with nitric acid and tested for copper by electrolyzing, using the point of platinum wire as cathode. In this way any possible loss of copper by incomplete precipitation in either of the electrolyses is prevented. If any copper is found in this check test it should be dissolved from the platinum wire, added to the solution obtained by dissolving the copper from the small electrode, and the electrolysis repeated in order to get the true weight.

In case a quantity of copper too small to be weighed is obtained its identity as copper may be most easily established by electrolyzing it onto the point of a platinum wire as described above. In these electrolyses with the platinum wire as cathode the current must, of course, be kept low in order to obtain satisfactory deposits. If this precaution is observed the deposit on the platinum wire will be of a brilliant red color and easily distinguishable as copper. If the deposit is brownish or blackish its identity as copper may be established by the green flash when the point of the wire is held in the colorless flame of the Bunsen burner, particularly if the wire has been first dipped in hydrochloric acid. Nitric acid must not be used, as nitric acid itself will give a green flash in the Bunsen burner flame.

The reagents used in the above process should all be tested as to freedom from copper. The water used should be doubly distilled and, at least the second time, from glass. All utensils should be cleaned by boiling in nitric acid. Care must also be taken to conduct the operations in rooms free from dust which might possibly contain copper.

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PLATE 6

Fig. 1.—Root system of corn plant injured by 0.1 per cent of copper added as copper sulphate to the soil.

Fig. 2.—Normal corn root grown in similar soil containing no copper. (Photos by G. F. Freeman.)